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HOST-PARASITE RELATIONS AMONG THE DIGENETIC TREMATODES*

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The subject originally suggested for this paper was host-parasite relations among the trematodes. I suggested limiting it to the digenetic trematodes and later came to a realization that even as limited the subject was much too broad. Therefore, I am limiting the discussion chiefly to those activities of the digenetic trematodes which, at various stages in the life-cycle, bring them into their hosts and the routes taken by them to reach the sites of infection. Some attention will be given to the routes of exit of certain so-called larval stages.

Any reasonably complete treatment of the subject of host-parasite relations as conceived by the late Dr. Robert Hegner for PROTOZOA would include such topics as host-parasite specificity, immunity, passive or acquired immunity, age immunity, effects of parasite on the host and of the host on the parasite, as well as symptomatology and pathogenesis. Obviously such a treatment is not feasible in a symposium paper. The number of hosts involved in the life-cycle of any digenetic trematode and the wide variations in details and even in patterns of life-cycles make it difficult to present broad generalizations regarding the host-parasite relations of this group of worms. It is no longer possible to think of the relations existing between the various stages of the sheep-liver fluke and its hosts as representative for digenetic trematodes.

In their life-histories, DIGenea employ either two or three hosts. The egg-laying generation lives in a vertebrate and their eggs must reach water or damp soil where development may continue to the miracidial stage within the egg, or where the miracidium, already developed in the egg before leaving the host, may remain alive. No miracidium can withstand complete desiccation.

In order that the life cycle may proceed it is necessary for the miracidium, with perhaps a single exception, to get into the body of a mollusk, such as an aquatic or terrestrial snail, or a bivalve. Entry is passive, if the egg is eaten by the mollusk. This is the usual method of entry for miracidia of those trematodes which produce small eggs. Examples are the HETEROPHYIDAE, OPISTHORCHIIDAE, BRACHYLAE-MIDAE, and PLAGIORCHIIDAE. It should be noted that both terrestrial and aquatic snails are avid eaters of feces and that they gather around the dung of vertebrates to feed. The entry into the molluscan host by most miracidia of medium or large size

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is brought about by their active penetration through the soft mucous surface of tentacles, head-foot organ, the mantle, or possibly of the lining of the respiratory chamber into which they have been observed to disappear.

The act of penetration into the tissues may require only a few seconds or possibly as much as a minute (Najim, Anne Vander Woude, unpublished). Penetration is accomplished by the joint action of muscular movements, activity of cilia located on the epidermal plates and the lysis of host cells, or perhaps of the mucoproteins constituting the intercellular cements by the secretion discharged from the penetration glands. These secretions probably contain hyaluronidase which has been found by Levine et al. (1948) in cercariae possessing penetration glands. The actual process of penetration is probably the same whether the miracidia attack a soft external surface of the host or the epithelial lining of the digestive tract by those emerging from ingested eggs.

Whether the small miracidia which are ingested while in the egg come into contact with suitable molluscan hosts appears to be largely a matter of chance, reduced somewhat by the fact that in general the final hosts and suitable molluscan hosts usually inhabit niches of the same habitat or come together at the same habitat for longer or shorter periods of time. Even so, the hazards involved in making connections with suitable mollusks are truly enormous, a risk which is compensated for by the great number of eggs produced by the worm and by the large output of cercariae released by the sporocysts or rediae which develop in the molluscan host resulting from the successful establishment of a miracidium.

For bringing about contact between free-swimming miracidia and suitable mollusks various factors have been suggested, including responses to gravity, light, and to chemical agents. Probably all play their parts. Suitable responses to gravity and light tend to bring the miracidium into the stratum of water or to the substratum where the molluscan host lives. Concerning response to chemical stimuli, some very definite statements have been made. For example, Faust (1924) stated concerning miracidia of *Schistosoma japonicum*, "if the susceptible mollusk is present a reaction on the part of the larvae is observed as soon as they come within a few millimeters of the snail . . . in order to cause this reaction the mollusk must pass through the subsurface stratum in which the miracidia are congregated. It seems probable, therefore, that snails which usually live some distance under the surface of the water would not ordinarily be subject to infection, even if they were capable of stimulating an attack by the miracidium. Once a miracidium of *Schistosoma japonicum* has come within stimulating range of the proper snail it makes a beeline drive for that snail and attacks it at the point of contact."

Observations by several students in my laboratory using other species have repeatedly led to the conclusion that the range of the stimulating effect of the snail upon the miracidium is very minute. These students are agreed that under laboratory conditions trial and error are much more important than chemotactic response in bringing the miracidium to the proper snail. Frequently, to the exasperation of the observer, the miracidium, after repeated contacts with the soft external surface of the snail of suitable species and age, swims away, sometimes to return and penetrate later. These students usually have employed very small quantities of water as the medium for the infection experiment.

Nevertheless, a chemical stimulus operating over very short distances probably

explains the selection of the mollusk as one suitable for penetration. Age of the mollusk is probably a controlling factor for many species of miracidia. Among the blood flukes, SCHISTOSOMATIDAE and SPIRORCHIIDAE, and in *Paragonimus kellicotti* (TROGLOTEMATIDAE), also in *Clinostomum marginatum* (CLINOSTOMATIDAE), many experiments have shown that young snails, from a few days to a few weeks, are most readily penetrated. Miracidia of certain species rarely or never penetrate snails over two to three months of age. This limitation does not appear to exist for certain other species.

Arrived within the tissues of a suitable mollusk, the miracidium migrates to the organ or tissues suitable for its development into the sporocyst stage. In *Spirorchis parvus*, Wall (1940) found the mother sporocyst in tissues of the mantle of the snail, a region often overlooked in the search for this stage. In *Schistosomium douthitti* the mother sporocyst occurs in lymph spaces about the gut in the head and neck region of the snail and immediately dorsal to the esophagus and cerebral ganglia. In *Schistosoma mansoni* Olivier and Mao (1949) reported on the locations of 95 mother sporocysts as follows: 30 near the surface of the head-foot, 26 on the mantle, 25 on the tentacles, 10 in the pseudobranch, 3 on the respiratory membranes, 1 near the brain, and 1 on the surface of the stomach. None was reported from the liver. These considerable variations in the site of the mother-sporocyst of one species and in one species of snail is of great interest and indicate the possibility of less fixity of location than is often presumed. It is, however, of special interest to note that the mother sporocyst in most species normally occurs elsewhere than in the liver (digestive gland) which is by far the most common locus for daughter sporocysts and rediae.

Why mother sporocysts choose these preferred locations or why their progeny, daughter sporocysts or rediae, generally migrate to organs different from that occupied by the parent remains a matter of speculation. Again one may invoke the magic word chemotaxis; but that is only another way of saying that we do not know the answer. Perhaps, it may be assumed that the preferred location for each of the generations in the molluscan host provides the necessary physical and chemical requirements for maintenance, growth and reproduction. Thus far no adequate analyses of these physical and chemical conditions have come to my attention. Quite a different idea may be worth consideration, viz., that the migration of the younger generation from the locus occupied by the parent may represent a change of host that might have occurred early in the evolutionary history of DIGENEA. In such a concept, change of locus in the molluscan host has resulted in a reduction in the actual number of hosts involved in the life-cycle, and in consequence has increased the possibility of survival by a reduction in the hazards involved. This view is wholly speculative and is not subject to proof.

The number of generations of sporocysts or of redia is not known with accuracy for many of the species whose life histories have been worked out in considerable detail. One difficulty is that the generations in the mollusk, subsequent to the mother sporocyst, rarely have sufficiently striking morphological characters to permit distinctions between them. Ameel (1934) found in *Paragonimus kellicotti* two generations of rediae marked by structural differences, but occupying the same portion of the snail's liver. For *Megalodiscus temperatus* Anne van der Woude (unpublished) was able to distinguish at least three generations of rediae, each

occupying a distinctive location in the snail. For *Clinostomum marginatum* Edney (1950), on the basis of life history experiments, postulated numerous generations of rediae which keep the infection going in the snail even into the third year. These generations were, however, indistinguishable on the basis of morphological characters.

Within the parent, whether sporocyst or redia, eventually a generation of cercariae is produced. These are the larvae of the egg-producing generation. What factors operate to cause the embryo to develop into cercariae rather than into sporocysts or rediae are as yet unknown. It has been shown by Chen (1937), van der Woude (unpub.) and Churchill (unpub.) that the embryos of all generations in the digenetic trematodes studied undergo parallel development. In *Fasciola hepatica* cercariae and rediae have been observed within a parent redia by Thomas (1883) and Krull (1933), and in *Clinostomum marginatum* by Edney (1950).

Before entering the vertebrate host the cercariae must leave the parent and make their way through the host's tissues and lymph channels to the exterior where, in most families, they escape into the water to swim or to creep about for a longer or shorter period. What causes them to leave the parent? Since many sporocysts and rediae are not provided with a birth pore the cercariae must escape from the parent under their own power. One of my students (Kruidenier unpublished) has suggested that they escape because they are no longer adapted for the conditions existing in the parent. They emerge from the parent in varying stages of development, some species long before they have attained full cercarial stature. These linger for some days in the tissues of the host to complete their development. Others are practically at full stature on leaving the parent, and for them the period of wandering in the host's tissues is usually very short.

Among the SCHISTOSOMATIDAE and the SPIRORCHIIDAE the contents of the first pair of penetration glands are used in getting out of the snail; for the number of penetration glands has been shown (Price, 1931; Wall, 1940) to be one less in cercariae which emerge naturally than in those secured by dissecting the snail. A similar loss of contents of a pair of penetration glands has been observed by Fischthal (unpublished) in the rhopalocercous cercariae of certain species in the family GORGODERIDAE. Such loss of gland content has not been observed for the many species of cercariae in the superfamily STRIGEOIDEA. Cercariae of some families do not possess penetration glands. Nevertheless, they successfully make their exit from the molluscan host.

I am assuming that the normal life-cycle of DIGENEA involves three hosts and that all instances in which the number of hosts is two have been derived from the three-host life-cycle by the elimination, usually, of the second intermediate host. This probably holds for the trematodes whose cercariae encyst on a substratum and are then ingested by the final host. These include the FASCIOLIDAE, NOTOCOTYLIDAE, PRONOCEPHALIDAE, and PARAMPHISTOMATIDAE and for those species which encyst within the mollusk without having ventured outside.

For the blood flukes, SCHISTOSOMATIDAE and SPIRORCHIIDAE, which likewise employ but two hosts in the life-cycle, an explanation of the reduced number of hosts is less readily found. Szidat (1931) hoped for clarification of this problem in further studies on the development of strigeids, in some of which as, for example, *Apatemon gracilis* (Rud.) the cercariae develop into the metacercarial stage in blood

vessels of leeches. Despite the numerous life-history studies of recent years on strigeids and the blood flukes, little more light has been thrown on this problem. However, instances of progenesis, i.e. the precocious development of trematodes to the egg-laying stage in intermediate hosts, have been found. Indeed, in certain species of *Proterometra* (AZYGIIDAE) the reproductive organs of the cercaria may be so far advanced as to have eggs in the uterus. It is conceivable, therefore, that blood flukes by a process of progenesis may in the remote past have eliminated the original final host by coming to the sexual condition in the intermediate host.

This concept is not at present subject to proof and may never be proved. However, the observed fact may be submitted that the cercariae of the closer relatives of the blood flukes, viz. the CLINOSTOMATIDAE and the STRIGEOIDEA all penetrate into second intermediate hosts, as do certain of their somewhat more remote relatives in the subfamily BRACHYLAEMINAE and in the BUCEPHALIDAE.

A reduction from the normal three-host life-cycle is accomplished in the LEUCOCHLORIDIINAE (BRACHYLAEMIDAE) by the encystment of the tailless cercariae within the parent sporocyst. In the genus *Plagioporus* (ALLOCREADIIDAE) cercariae of *P. sinitsini*, according to Dobrovolsky (1939), encyst within the colored sporocyst and the latter with its burden of metacercariae emerges from the snail and moves actively. The eating of this motile sporocyst by small fishes completes the transfer of the metacercariae to the final host. So far as known, all other species of *Plagioporus* have actively motile cercariae which must penetrate into second intermediate hosts. In the GORGODERIDAE the rhopalocercous cercariae encyst within the tail chamber (Fischthal, unpublished) and thus appear to require only two hosts, whereas the macrocercous cercariae belonging to the subfamily GORGODERINAE develop into metacercariae in second intermediate hosts and come to the sexual condition in a final host.

The significance of the second intermediate host to the metacercaria is a matter of wide variation among the species employing such hosts. Certain metacercariae are infective at once, or almost at once, after the accomplishment of encystment. These include some of the ECHINOSTOMATIDAE. For such species the intermediate host contributes stimuli for encystment, provides protection and a convenient vehicle for transportation into the final host. Little or no development occurs in these metacercariae and correspondingly small demands are made on the host for nutrition.

In contrast to these are the many species whose metacercariae require a period of some days or even weeks or months for sufficient development to make them infective for the final host. As examples one may cite *Pneumonoeces medioplexus*, which requires a minimum of six days for development to the infective stage in the dragonfly (Krull, 1931), and *Paragonimus kellicotti* requiring 46 days in the crayfish (Ameel, 1934). *Clinostomum marginatum* has been found by Edney (unpublished) to require several months for the development of the large infective metacercaria which is sexually mature. Among the STRIGEOIDEA the researches of Szidat (1924) have demonstrated considerable vacuolation and liquifaction of the tissues of the penetrated cercariae followed by a reorganization of many of the organs and the appearance of new ones. These extensive changes require several weeks.

In general, the metacercarial stage is spent within a cyst, whether the parasite is attached to a substrate or lives within a second intermediate host. In the first

instance the cyst, produced by the hardening of materials discharged from specialized gland cells, interposes a more or less effective barrier to the ingress or egress of water and of salts, and must be considered to be a protective mechanism of considerable effectiveness. Most metacercariae which live for a time in second intermediate hosts provide themselves with protective cysts, usually thinner and more delicate than those surrounding cercariae encysted on a substrate. These cysts are derived also from secretions of cystogenous gland cells. Vertebrate hosts commonly respond to the presence of these parasites by walling them off within cellular investments which may be delicate or thick, sometimes consisting of many concentric layers of cells derived chiefly from connective tissue elements. Often in fishes these coatings are hardened to a pearly or flinty consistency by the deposition of salts.

Exceptions to encystation of metacercariae are found in the diplostomula which inhabit the humors of the eyes of fishes, or live within the lens capsule, also in those which live in the ventricles of the brain or the neural canal of the spinal cord. In these situations there is a dearth of connective tissue cells from which an investment of host cells could be made. Further exceptions are found in the mesocercariae of *Alaria mustelae* and *A. marcianae* which live in lymph spaces between the tissues of tadpoles, frogs, and such frog-eating snakes as *Thamnophis* and *Natrix*. The cercariae which develop into the diplostomula, referred to above, are not provided with cystogenous glands. The continued activity of the young worms or the lack of suitable connective tissue elements in the sites chosen do not permit investment by host cells. For similar reasons the cercariae of BRACHYLAEMINAE, which creep into the external opening of the kidney of land snails or slugs and thence make their way through the cavity of the kidney and thence into the cardiac chamber through the cardio-renal duct, do not encyst, nor are they invested with cells of host origin (Ulmer, unpublished).

In addition to the routes of entry already presented, are those of ingestion and of inhalation of cercariae by the second intermediate host. The movements of the large and sometimes colored tails of certain species of cercariae simulate the movements of mosquito and other small insect larvae. Such cercariae are often ingested as prey. In the host's gut the cercarial body actively penetrates through the tissues, comes to rest in a suitable situation and encysts. Thus some species of GORGODERIDAE, some ECHINOCHASMINAE (ECHINOSTOMATIDAE) and probably some of the ALLOCREADIIDAE get into their final hosts which may be such predatory insects as dragonfly larvae, *Sialis*, aquatic beetle larvae, crayfishes and even some small fishes. Some small cercariae are eaten. The tailless cercariae of *Triganodistomum mutabile* (Cort) are eaten by the commensal oligochaete, *Chaetogaster*, and by a triclad turbellarian (Wallace, 1941). The minute cercariae of *Pneumonoeces medioplexus* are swept into the rectal-branchial chamber of certain dragonfly nymphs by the currents of water caused by the respiratory movements of the abdomen (Krull, 1931).

Certainly, the devices employed by cercariae to gain entry into their hosts are varied and ingenious. To the observer, cercariae appear to display a detailed knowledge of the feeding and other activities of the appropriate hosts. It must be remembered that in general each species of cercaria is adapted, probably, to the biochemical environment of a rather limited range of hosts. There must be considerable loss

of parasites by failure to make connections with suitable hosts or by being eaten by unsuitable hosts.

Entry of the encysted metacercariae into the final host is by way of the mouth. This certainly holds for all species whose cercariae encyst upon a substrate and also for those species which employ second intermediate hosts.

Excystation of metacercariae is accomplished in the final host probably by the digestion of the cellular investment by means of proteolytic enzymes. Whether the inner portion of the cyst which is of parasite origin is acted upon by the digestive and muscular activity of the parasite is not known with certainty. However, the extreme difficulty in bringing about release of certain metacercariae by means of enzymes, used singly or in series, leads to the belief that in these species the parasites may release themselves when stimulated by the proper ions at a suitable temperature. For instance, Ameel (1934) was fully as successful in bringing about excystation of *Paragonimus kellicotti* in physiological salt solution as in activated gastric or pancreatic fluids, used singly or in series. The cyst of this species has no cellular elements and appears to consist wholly of substances derived from the parasite.

Following excystation the young worms move to the organ of choice. Their movements apparently are not infallible; for the literature is well sprinkled with reports of the finding of trematodes in unusual situations. Nevertheless, each species appears to have its preferred location where it can usually be found. The factors which operate in the choice of habitat are not known. Some of them can be conjectured. In some instances, it probably is safe to seek an explanation in the concept of chemotaxis. Some of the routes followed are so devious as to require an assumption of a series of chemotactic responses which lead the young worm from the place of excystation eventually to the normal location. Such trematodes as *Clonorchis sinensis* and *Opisthorchis felineus* can follow either a chemical trail or an actual physical track along the common bile duct. Is it chemotaxis, or a series of chemotaxes, by which *Paragonimus* is led through the gut wall into the coelom, thence through the diaphragm into the pleural cavity and finally into the lungs? Or is it a series of taxes including chemotaxis, plus responses to tactile stimuli, oxygen gradients, etc. which bring the young parasite to the lungs? A temperature gradient can hardly be involved. Someone may claim that the behavior of the worm is instinctive, an explanation that still leaves us in the dark.

In contrast to the devious route followed by *Paragonimus* the young *Pneumonoeces*, excysting in the stomach of the frog, ascends the esophagus to the mouth, then passes through the glottis into the lung where it passes its adult life. Metacercariae of the family OCHETOSOMATIDAE (RENIFERIDAE), excysting in the gut, seek out their respective habitats in snakes as follows: *Zeugorchis*, *Dasymetra*, and *Stomatrema* in the esophagus, *Ochetosoma* (*Renifer*) in the mouth, *Pneumatophilus* in the trachea, and *Lechriorchis* in the lung (J. D. Goodman, personal communication).

In the family FASCIOLIDAE, *Fasciola hepatica* and *Fascioloides magna* seek out the liver, but *Fasciolopsis buski* remains in the intestine. In the family TROGLO-TREMATIDAE, *Paragonimus* localizes in the lungs of mammals, *Sellacotyle* in the intestines; *Trogloitrema acutum* occupies the frontal and ethmoidal sinuses of mustelids, but metacercariae of *Collyriclum* penetrate to the subdermal tissues of birds

where they live in cysts under the skin. Each cyst is provided with a pore to the outside through which eggs escape.

In fishes such organs as swim bladder, pyloric ceca, urinary bladder (if one exists), and mesonephric ducts are all occupied by digenetic trematodes. In frogs urinary bladder, kidney tubules, even Muellerian ducts and oviducts may be occupied by gorgoderid worms.

In young gulls the bursa Fabricii is a favorite habitat for certain strigeid worms, and the ceca of birds or mammals are the habitat for certain BRACHYLAEMINAE. However, the LEUCOCHLORIDIINAE, belonging also to the BRACHYLAEMIDAE, occupy the rectum and bursa of birds.

The blood flukes, as indicated earlier, have only two hosts in the life cycle, a mollusk and a vertebrate. After a brief sojourn in the water, the cercariae enter the vertebrate host by penetrating actively through the surface of the body. En route to the blood vessels of the preferred organ, they pass through the heart, and the schistosomes of mammals go by way of the pulmonary artery to the lungs. From the lungs most of them go to the liver where there is considerable development. The route from lungs to liver may be by way of the blood vessels, as claimed by certain workers; but certain others have evidence to indicate that they may bore out of the lungs into the pleural cavity, penetrate through the diaphragm and thence into the liver (El-Gindy, unpublished). There seems to be agreement that a considerable portion of these young worms reach the liver and that, following a period of growth and sexual development, most of the young males and females make their way to preferred sites in the venules of the lower alimentary canal or urinary bladder, depending on the species. Some worms lose their way at some stage of the journey, and are recovered in unusual situations. This is particularly true in cases of very heavy infection. So far as I am aware, the route of migration of the blood flukes of turtles has not been described. According to Wall (1940) the blood flukes of turtles are always found in the arterioles or in the heart. This is in contrast to the blood flukes of mammals and birds which always occupy venules. No adequate explanation for this difference of habitat has been offered; but one may hazard the guess that in their respective habitats the blood flukes of these two families probably find the oxygen tension at the optimum.

Cercariae of schistosomes select their final hosts apparently in response to thermal stimuli. It is well established that the cercariae of avian schistosomes penetrate into mammals as readily as into birds. The converse may be true, but available evidence is not at hand. However, the cercariae of avian and mammalian blood flukes do not penetrate into cold-blooded vertebrates, nor do the cercariae of blood flukes of turtles penetrate into birds or mammals.

In the course of its complex life-history, a digenetic trematode encounters great variations in the osmotic pressures of the media in which it must live for longer or shorter periods of time. The miracidia and the cercariae which emerge into freshwater are subjected to low osmotic pressures; those emerging into brackish water or undiluted seawater encounter high osmotic pressures.

When miracidia penetrate into mollusks they encounter relatively high osmotic pressures, higher than that of freshwater, lower than that of seawater. Metacercariae which are encysted on a substrate are protected from an adverse osmotic pressure by the relatively impermeable cysts. Within the second intermediate

host and within the final host, the metacercariae and the adult worms, respectively, find osmotic pressures and biochemical environments very different from those on the outside of the host to which their remote free-living ancestors must have been well adapted.

In general, it can be stated that species of DIGenea are well adapted to the various conditions imposed upon them during the various stages of the life-cycle, assuming that events follow their normal course of media and hosts. Failure of a species to establish itself in an unusual host probably results from a lack of ability to make adjustments to the somewhat different biochemical conditions to which it is now exposed. Or, in some cases the worms may not be able to withstand the adverse effects of immune mechanisms which the host brings to bear on an unusual parasite. The person studying life-histories by the experimental method encounters many unexplained blocks to his efforts. He may not appreciate that the chemical environments provided by one species of turtle, for example, are different from those provided by another turtle species from the same stream. The parasites may survive for a time, but too frequently disappear from the host before attaining sexual maturity.

In conclusion let me state that, although it is possible to make certain broad and loosely defined generalizations regarding the host-parasite relations or adaptations of some families of DIGenea, those who have been engaged in unravelling trematode life-histories have come to realize all too well how many of their generalizations fail when they are applied to a particular species. Each species seems to play the game of parasitic life within the broad rules laid down by, and for, its family; but it appears to have developed within this code its own special rules and regulations, its own deviations from what we poor humans assume to be normal and regular for the family.

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HOST-PARASITE RELATIONSHIPS IN CESTODE INFECTIONS, WITH EMPHASIS ON HOST RESISTANCE*

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For adequate coverage of host-parasite relationships, attention must be directed not only to the many relations between host and parasite during infection, but also during the period when transmission takes place. In addition, the fascinating problems of host-parasite specificity and host-parasite adjustments should be considered. Therefore, by necessity, the present discussion of these relationships is quite incomplete, since the major emphasis will be on host resistance. As a matter of further expediency, no attempt will be made to review all of the literature, but rather selected references will be used for illustration. These deal chiefly with *Hymenolepis* in laboratory animals, not because of a desire to impart undue importance to this species, but because our knowledge of resistance to this cestode is fairly complete. This is no doubt due to the fact that it is the exception among cestodes, having in the same host a parenteral phase (cysticercoid) and an intestinal lumen phase (adult) permitting the study of all types of resistance. There is an added advantage of limiting this discussion for the most part to one host-parasite combination, since the various relationships are quite involved.

HOST RESISTANCE

It is customary to subdivide resistance into 2 main types, natural or innate, and acquired.

NATURAL RESISTANCE

The obstacles that must be met by the cestode at the time of invasion of a host for the first time constitute the natural resistance of the host. This type of resistance which is inherited is due in part to the nature of the host without respect to ancestral relations with the parasite, or it may have been built up during the course of evolution as a protection against infection. It may be quite variable in degree depending on the host involved. There may be differences in degree of natural resistance to a given cestode between species, between races or strains within the same species, and even between individuals of the same race or strain.

Species Resistance (differences in susceptibility of various species).—A given species of cestode infects normally only certain species of hosts. However, the mere absence of infection is not proof of species resistance, as exposure to infection may be lacking. Therefore, experimental studies, such as those with the mouse strain of *Hymenolepis* in various rodents by several investigators, give the best evidence for this type of resistance (Shorb, 1933; Hunninen, 1935b; Larsh, 1944a, 1946c). After similar test infections, hamsters and albino mice showed significantly more adult worms than rats, deer mice, and guinea pigs. Thus on this basis alone, the hamsters and white mice may be considered the most suitable, or

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normal, hosts for development of the adult worms, and may be said to possess the least species resistance of the hosts tested. It follows that the rats, deer mice, and guinea pigs are unsuitable, or abnormal, hosts, as a result of their strong species resistance. This resistance was absolute in the case of the guinea pigs, but only partial for the rats and deer mice. Partial species resistance may manifest itself in a number of ways, so that criteria other than the number of worms that establish would need to be considered in thorough comparisons of hosts. For example, the cestode may develop in large numbers but not live its normal life span, as shown by the worms in the hamsters in the present case. It is not always easy, therefore, to decide which host is most normal. White mice have been used in the majority of studies on *H. nana*, because of the ease with which they can be raised and handled in large experiments.

Comparisons were made in the above studies of the percentage development of cysticercoids following a test infection in all of the 5 rodent species except the hamsters. A somewhat similar percentage development occurred in the 4 hosts. This loose host specificity of the cysticercoids, as compared with the rather strict specificity of the adult worms, may have evolutionary significance favoring the view that the direct life cycle is more recent than the indirect, which requires an insect intermediate host. Recent support of this idea has come from studies of the indirect cycle in adult grain beetles, *Tenebrio molitor*, in which Bailey (1950) concluded that there is better adaptation of this parasite in the indirect cycle.

Racial or Strain Resistance (differences in susceptibility of different races or strains of the same species).—A survey by Otto (1936) showed that Negroes have a somewhat lower infection rate of *Hymenolepis nana* than whites. There is experimental evidence to support the view that significant differences in susceptibility to this cestode exist among members of the same host species. For example, after a test infection of three strains of the mouse species, *Mus musculus*, similar numbers of cysticercoids and adults were observed in white mice, and in wild house mice, but significantly fewer of both stages were recovered from dilute brown laboratory mice, the C57 strain (Larsh, 1944a). Since the mice of the dilute brown strain are of the same species as the other 2 hosts, their resistance may be termed strain resistance. Additional comparisons of the white and wild mice showed that 11-day worms were much larger in the wild mice. This indicates less interference with the growth of the worms and suggests that the wild mice are a more suitable host than white mice.

Individual Resistance (differences in susceptibility of individuals of the same race or strain).—In quantitative studies of natural resistance, there are often differences noted between individuals, even those born of the same litter. While this points to individual differences in susceptibility, it is difficult to prove, since certain technical errors can not be ruled out. There is, however, one type of individual resistance, called age resistance, for which there is ample experimental evidence. Several studies on the host-parasite relationships have shown that white mice of the same strain less than about one month, or more than about five months, of age have striking resistance to initial infection with *Hymenolepis* (Woodland, 1924; Shorb, 1933; Hunninen, 1935c; Larsh, 1944c, 1950b). Sandground (1929) considered the development of age resistance in old members of a strain an abnormal host relationship, and others have given support of this view (Porter, 1935). With

this in mind, it is worth pointing out that wild house mice apparently do not develop such resistance to *Hymenolepis*, since those tested at 5 to 6 months of age were as susceptible as those of 2 to 3½ months (Larsh, 1944a). Thus, the wild mice may be the true normal host. This is supported by the above comparisons showing that the worms grow faster in them than in white mice, previously considered the best host.

There are many other studies reported which show that the phenomena of species resistance, racial or strain, and individual resistance operate against other cestodes (Culbertson, 1941).

The Mechanism of Natural Resistance.—If a parasite is equipped to enter a host and its development is hampered, unsuitable physical and/or chemical conditions, or unsuitable food, may be responsible. However, even in the presence of suitable stimuli for development, the host may possess certain non-specific defenses which serve as barriers to such development. Mechanical features, natural secretions, normal body fluids, and various cellular reactions, to mention only four, have been shown to be effective defenses against invasion by certain organisms. Because some of these variables are difficult, if not impossible, to control by experimental methods, the exact mechanism of natural resistance may never be learned. However, hypotheses based on factors known to operate in some cases have been proposed, which give a better understanding of this protective mechanism.

The most obvious factor to account for differences in certain types of natural resistance is the genetic constitution of the host. Dramatic proof of this is seen in the artificial production of resistance through experimental breeding. There is danger in stressing this factor, however, since the make-up of the parasite, likewise subject to change, may be entirely overlooked. That such changes in the parasite may be important in certain cases is illustrated by Shorb's (1933) "physiological strains" of *Hymenolepis*, one better adapted to rats, the other to mice. There also is some evidence that there may be more than a single mouse strain (Larsh, 1943d). Such strains may be rigidly adjusted to one host, as was shown by the failure to affect the percentage development of cysticercoids of the mouse strain in rats and mice, after many transfers in rats (Larsh, unpublished data). The recent cytological studies of Jones (1945, 1948), describing the chromosomes of this and other cestodes, have an interesting bearing on this matter. His evidence of inherent genetic instability of this species might explain the emergence of such strains. Thus, the failure to change the infection rate might indicate the elimination, by selection, of the original non-adapted genotypes. In any event, it would seem advisable not to attribute physiological variation in a strain solely to environmental differences supplied by different hosts, since such variation also may be due to the selective effect of such environments.

Although the hereditary factor undoubtedly plays an important role in certain types of natural resistance, very little is known of the bases for the resulting resistance, or, in other words, how heredity affects the mechanism of resistance. Most of the knowledge concerning this mechanism has resulted from studies of individuals of a single strain of host, in which hereditary effects are minimized. The examples here are from studies of white mice infected with eggs of the mouse strain of *Hymenolepis*.

The age of mice, as mentioned above, has been shown to be a factor in their natural resistance to this cestode. The strong resistance of very young mice probably is due in large measure to the small size of the intestine, which, as first suggested by Hunninen (1935c), serves as a mechanical obstruction to the normal hatching and penetrating activities of the oncospheres. Larsh (1943a) gave strong support to this view by artificially increasing the intestinal size of the young mice with hormone injections, which altered their resistance so that they harbored about the same number of cysticercoids as mice 2½ months old, the most susceptible age for infection. This strong resistance of young animals to helminthic infection is unusual, since such resistance usually increases with advancing age.

The resistance of old mice to *Hymenolepis* infection has not been explained satisfactorily. It is not the result of increased speed of development of acquired resistance compared with that in younger animals, as suggested for the resistance to dog hookworm (Cort and Otto, 1940), since cysticercoids of an initial infection are not prevented from developing (Bailey, 1950). However, certain conditions have been shown by experiments on mice to interfere with age resistance. Splenectomy of young mice causes a progressive anemia which is correlated with the failure of the animals to develop usual resistance when they become old (Larsh, 1944c), and injections of thyroid extract, and also alcohol, bring about a loss of such resistance (Larsh, 1950b, and unpublished data).

This method of modifying the host, by subjecting it to various conditions, has been used in other studies to demonstrate factors which may influence the mechanism of natural resistance. By this approach, a considerable number of factors have been tested, but only 3 have been shown to cause a decrease in the usual numbers of *Hymenolepis* found after infection, *viz.*, generally deficient diet (Shorb, 1933), reduced body temperature (Larsh, 1945b), and an intercurrent infection with *Nippostrongylus muris* (Larsh and Donaldson, 1944). On the other hand, 4 different factors are known to cause an increase in usual numbers of this cestode after infection, *viz.*, a protein-deficient diet (Larsh, 1950c), alcoholism (Larsh, 1945b, 1946a), pregnancy (Larsh, 1949), and decreased intestinal emptying time (Larsh, 1947a).

Diet as a factor in modifying host resistance may exert its influence in a number of ways. Thus, it is noticed that a generally deficient diet may cause fewer worms to develop, hence appear to increase host resistance, as shown in Shorb's work in which adult worms were counted. This is more apparent than real, however, as the effect was shown to be directly on the worms, slowing their growth and causing expulsion prior to maturity. On the other hand, a protein-deficient diet is listed as causing an unusual increase in numbers of parasites, or decreased resistance. In this case, in which cysticercoids were counted, the beneficial effect on the parasite probably was indirect by interfering with the normal defensive mechanisms of the host. This difference in dietary effect on resistance, which is related to the location of the parasite, agrees with Chandler's (1948) views. Therefore, in evaluating the effects of such debilitating and stimulating factors, the parasite as well as the host must be considered.

Since the cysticercoids of an initial infection have been shown to stimulate only a mild host tissue response, which is not sufficient to inhibit their growth

(Bailey, 1950), factors having to do with hatching and penetration of the oncospheres into the villi would seem to be most important in this resistance. It is interesting, therefore, that incubation of the eggs in fecal pellets stored in tap water brought about an unusually large percentage development (Larsh, 1943c). Since mechanical rather than enzymatic action is involved in the hatching of these eggs (Joyeux, 1920; Kiribayashi, 1933), it is conceivable that such incubation, through bacterial action, might weaken the egg shells making hatching easier than usual. In any event, this raised the question whether the eggs isolated from fresh feces for experimental infections encounter a mechanical disadvantage in animals known to have a rapid intestinal emptying time. Accordingly, this factor was studied as to its effect on natural resistance.

The effect of intestinal emptying time was demonstrated directly by the use of drugs to slow the intestinal motility (Larsh, 1947a). The increased percentage development of cysticercoids associated with this change presumably resulted from delayed passage of the eggs, which allowed more of the oncospheres to hatch and penetrate the villi than under normal conditions of rapid intestinal movement. Support of this suggestion was brought out in one of the studies mentioned above, in which increased intestinal length, produced artificially, was correlated with an increased percentage development in young mice (Larsh, 1943a). As noted above, pregnancy of mice was found to reduce natural resistance to *Hymenolepis*, but the mechanism was not studied. The lowered resistance of chronic alcoholic mice must be due to a deficiency state produced by the reduction in food intake, since polyvitamin injections given with the alcohol prevented interference with the resistance (Larsh, 1947b). Both pregnancy and alcoholism may be found, in final analysis, to exert their effects on resistance through decreased intestinal motility, as this factor is known to accompany pregnancy and Vitamin B deficiency (Alvarez and Hosoi, 1930; Gross, 1924). These studies on intestinal emptying time, together with recent reports of the role of this factor in other host-parasite relations (Ackert and Ameel, 1947; Larsh and Hendricks, 1949; Larsh, 1950a), indicate that it may prove to be of fundamental importance in the mechanism of natural resistance to certain parasites localized in the small intestine.

The demonstration of these various factors which modify natural resistance opens the way for further study of the mechanism. In addition to the direct value of such studies in understanding resistance, they serve to increase knowledge of host-parasite relations, which may possibly lead to a better understanding of the evolution of parasitism.

ACQUIRED RESISTANCE

If a cestode possesses means of resisting the natural defenses of the host and, on this account, is successful in its attempt to grow and bring about an infection, deep-seated physiological changes may be produced in the host, which result in the development of acquired resistance. A similar, if less pronounced, development of resistance may follow deliberate parenteral injection of non-living cestode products. In both cases, the acquired resistance is termed active, since the body cells are stimulated to produce the resistance. As a rule, active resistance is developed rather slowly, but is highly specific and long-lasting. In instances where the protective elements developed in one host are transferred to another, the resulting resistance is called passive, as the body cells of the recipient are not

aroused in the process. Acquired resistance may be shown by the host by reduced numbers of worms that establish themselves after reinfection, by interfering with normal growth and reproduction of the worms, or by shortening the life of the infection. In general, the information concerning active and passive resistance is more complete than that for natural resistance, because the changes occur during the lifetime of the individual and are thus more easily studied under experimental conditions.

Active Resistance.—Many surveys show that *H. nana* in man is more common in the younger age groups, which may indicate that the adults of these populations are able to resist reinfection by the development of active resistance (Chandler, 1922; Spindler, 1929; Otto, 1936). It is not surprising, therefore, that early workers noted a striking reduction in *Hymenolepis* in experimental animals after reinfection (Grassi, 1887; Joyeux, 1925; Brumpt, 1933). Critical comparisons of resistance to cysticeroid development, however, awaited Hunninen's (1935a) quantitative methods. Several workers have emphasized the striking degree of resistance produced against this parenteral phase (Hunninen, 1935c; Hearin, 1941). In response to worm materials (antigens) released during infection, specific substances (antibodies) are produced in the body which have been detected by standard serologic procedures (Larsh, 1943b). Hearin (1941) showed that resistance to this cestode may manifest itself within 12 hours after infection and remain at a high level for several months, even after removal of the adult worms by treatment. His demonstration that the adult stage does not confer demonstrable resistance has been recently confirmed by Bailey (1950), in his studies on the indirect cycle. This demonstration agrees with that for *Taenia taeniaeformis* (Miller, 1932b), *Hymenolepis diminuta* (Chandler, 1939), and other cestodes (Culbertson, 1941). However, some workers have noted evidence of protection against infections produced by the adult worms (Stoll, 1935; Turner, Berberian, and Dennis, 1936). It may well be, as pointed out by Stoll (1948), that our thinking and experimentations on resistance to adult tapeworms have neglected the great importance of reinfection, which is Nature's substitution for *in vivo* multiplication. He gives examples showing that the differences in resistance could be purely a difference in the number of parasites that assault the host. Thus, future work on cestodes should test this point by giving repeated reinfections in order to determine what he calls the true "reactive potential" to the parasite.

Active resistance to *Hymenolepis* produced artificially by vaccination has been demonstrated in mice following the repeated use of fresh adult-worm antigen (Larsh, 1944b). In degree, the resistance produced by this deliberate inoculation of antigen was not as great as that following infection, perhaps because of slight changes produced during preparation of the antigen (Smith et al, 1948). Then, too, there is undoubtedly a quantitative difference, since during infection the amount of metabolic products released, the important source of antigen, would be greater than that released during vaccination.

Passive Resistance.—This type of acquired resistance, like active resistance, may occur naturally or be produced artificially. Young animals may receive from their mothers by natural means specific antibodies which offer protection against a particular organism, such substances having previously been elaborated in the body of the mother in response to antigenic stimulation. Larsh (1942) demonstrated

in mice that such protection to *Hymenolepis* is transferred *in utero*, and, in even greater degree, in the milk. As in all cases of passive resistance, the protection lasted only a short time after birth (in this case, 37 to 41 days), since such substances, however beneficial, are foreign to the body and soon eliminated. In less striking degree, the same phenomenon was demonstrated in the young of vaccinated mothers (Larsh, 1944b). The quantitative factor mentioned above probably accounted for this less striking effect on resistance.

The demonstration of resistance, acquired artificially, was accomplished by Hearin (1941) by injecting into previously uninfected mice serum which he had collected from donors given 2 *Hymenolepis* infections. While this was probably passive resistance, it was not proved as such since its duration in the recipients was not tested. This step is necessary to rule out the possible transfer of circulating antigens. These have been detected in the circulating blood soon after infection in at least one case and, if transferred, might produce active resistance (Bozicevich and Detre, 1940).

The above results on all phases of acquired resistance agree with those first demonstrated for a cestode by Miller (1931a and b, 1932a and b, 1935), and support the accepted view that such resistance to tissue stages is developed, and that the phenomena observed are similar to those acting against other infectious agents (Taliaferro, 1929, 1940; Culbertson, 1941; Larsh, 1945a).

The Mechanism of Acquired Resistance.—Since acquired resistance of a host is superimposed upon its natural resistance, proper controls must be used in experimental studies to show the effects of each. Thus, mice previously infected with eggs of *Hymenolepis* usually harbor no mature cysticercoids after reinfection, whereas previously uninfected controls show about 4 per cent development of the same batch of eggs. The difference in numbers of cysticercoids present in the 2 groups of mice, therefore, can be attributed to the action of acquired resistance. This resistance is so striking that reinfection is rarely observed. It has been shown, however, that certain debilitating factors interfere with the development and/or maintenance of this resistance, thus permitting reinfection. Factors demonstrated to have such an effect are an intercurrent infection with an intestinal bacillus, probably *Salmonella typhimurium* (Hunninen, 1936), an intercurrent infection with *Strongyloides* (Brumpt, 1933), alcoholism (Larsh, 1946b), and a protein-deficient diet (Larsh, 1950c). Since there is some evidence of a quantitative relationship between the amount of antigen released as a result of the first infection, and the degree of resistance to reinfection (Larsh, 1946b), study of such factors by the quantitative approach might yield information of more direct value in interpreting the mechanisms involved in their action.

While much has been learned about acquired resistance to *Hymenolepis* through experimental studies in mice, the mechanism of this resistance is not yet known. The above studies demonstrating that this resistance can be transferred passively show that specific antibodies are involved. On first thought, this suggests that the mechanism may be similar to that postulated to operate against certain tissue nematodes (Taliaferro and Sarles, 1939), in which the antibodies directly attack the invading organisms, and in some way cause them to be more easily encapsulated by cellular reactions. However, further consideration makes it difficult to apply this hypothesis, because resistance to *Hymenolepis* has been demonstrated within

12 hours after infection (Hearin, 1941), hardly enough time for sufficient antibody production.

New light was shed on the mechanism of resistance to cestodes by the study of Leonard and Leonard (1941) on *Taenia pisiformis* in rabbits. Seven days after inoculating viable onchospheres into the mesenteric vein of resistant animals and controls, they noted no significant difference in numbers of larvae beginning development in the liver. Therefore, the reduction in numbers of larvae seen in previously infected rabbits, as compared with the numbers in controls, is not due to factors operating against the organisms after they reach the portal blood. They thus concluded that in resistant rabbits the intestinal mucosa serves as an important barrier, by preventing onchospheres from reaching the blood stream. Bailey (1950) made a similar conclusion from recent histopathological studies of *H. nana*, after showing that very few onchospheres of a second infection were able to penetrate the intestinal wall. In both studies, it was shown that within the tissues of resistant animals larvae are destroyed by an accelerated host tissue response.

These studies permit speculation that the mechanism of acquired resistance to certain tissue cestodes may have two phases. The first in the intestine which prevents penetration of all but a few onchospheres, the other in the tissues which destroys most of the remaining organisms by an accelerated host tissue response. Both phases may be dependent upon the primary actions of specific antibodies with secondary cellular cooperation, as suggested for the mechanism against certain nematodes. However, considering the rapidity with which resistance to *H. nana* is produced, antibodies must play a more important role during the tissue phase. Proof of this hypothesis probably will demand more knowledge of the parasite's physiology. Also, a better understanding of the intestinal physiology of the host is needed to study such complex host-parasite relationships (Read, 1950).

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EVOLUTION OF ZOÖPARASITIC GROUPS IN THE PHYLUM NEMATODA, WITH SPECIAL REFERENCE TO HOST-DISTRIBUTION¹

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INTRODUCTION

In the NEMATODA the biologist is confronted with a well-defined group of organisms, relatively small as regards different structural types, but exceedingly numerous as individuals and successful in the most varied habitats. Traditionally in general zoology and parasitology texts—in the English language, at least—the group is considered an order or class, which, with orders or classes GORDIACEA (or NEMATOMORPHA) and ACANTHOCEPHALA, constitutes a phylum NEMATHELMINTHES. However, as the leading nematologist, B. G. Chitwood (1950c), has pointed out in a review of the historical placement of the NEMATODA in the animal kingdom, such a grouping has never had the support of serious students of invertebrate evolution. He has marshaled evidence in a succinct manner for the view, recognized by a growing number of zoologists, that the nematodes constitute the largest of a group of minor phyla—or classes within a rather broadly defined phylum, to which Hyman (1940, 1951a, b [in press]) has followed earlier German workers in applying the name ASCHELMINTHES Grobben, 1908.

Whether one wishes to consider the NEMATODA an independent phylum or an aschelminth class is of relatively minor importance (although it may be remarked that there is probably more justification in combining the ANNELIDA, ONYCHOPHORA, and ARTHROPODA into a single phylum than the so-called aschelminth classes). For present purposes I am considering the NEMATODA a phylum. What is actually important is recognition of the interesting evolutionary status of the NEMATODA. They represent one of a group of Nature's minor evolutionary experiments in metazoan organization and have exploited various possibilities of non-segmented, pseudocoelomatous, vermiform structure. They have never attained the remarkable diversity of the major animal phyla—in particular the MOLLUSCA, ARTHROPODA, and CHORDATA—presumably because evolutionary processes have been able to do only so much within the morphological limitations at the aschelminth level of organization. Moreover, they appear to lie on a relatively small branch of the evolutionary tree of the METAZOA, without direct relationship with any of the higher animal groups. This point of view has been graphically supported by Chitwood (1950c).

Nevertheless, the NEMATODA must, I feel, be recognized as one of the most successful of animal groups. These generally small worms teem in the soil, fresh water, and sea and inhabit the bodies of other living organisms, plant and animal.

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In point of numbers of individuals they are probably the most populous group of the METAZOA.

Parasitism³ is widespread and highly evolved in the NEMATODA, and in its pattern one encounters a somewhat striking difference from that of the other major worm phylum—the PLATYHELMINTHES—in which parasitism has also been developed to a high level. In the latter group, only two highly successful parasitic lines⁴ exist—the classes TREMATODA and CESTOIDEA. Both are well defined groups, with an evolutionary history long independent of that of the free-living class TURBELLARIA, from which they arose. There are, to be sure, a fair number of parasitic turbellarians, but these constitute minor groups that in some cases have become highly specialized, yet have radiated but little. One of these, the TEMNOCEPHALIDA, is sometimes (as by Dawes, 1946) recognized as a fourth platyhelminth class. Stunkard (1937) has reviewed in some detail the evolution of parasitic flatworms and has made some interesting generalizations. One of his points is that all gradations of parasitic adaptation exist in the flatworms. However, if one accepts the NEMATODA as a phylum, then his conclusion (p. 27) in regard to the PLATYHELMINTHES that “no other phylum presents such a continuous and complete transition from free living existence to parasitism” would be more appropriately applied to the nematodes, for in the NEMATODA there are numerous parasitic lines derived from free-living ancestors and of varying size and evolutionary success, from a parasitic order down to parasitic species within largely free-living genera. The interconnections of free-living and parasitic forms are thus far more evident and complex than in the PLATYHELMINTHES.

Other parasitic worm groups exist—the phylum ACANTHOCEPHALA, which is closely related to the PLATYHELMINTHES, and the phylum NEMATOMORPHA (or GORDIACEA), which is an independent aschelminth group, although recent evidence of uncertain validity has been used to link it intimately with the NEMATODA (Woodhead, 1950) and is touched on later in this paper. Within neither of these phyla is there a picture of complex evolutionary relationships between parasitic and free-living groups inasmuch as both are entirely parasitic and apparently monophyletic groups.

This complexity of interrelationship within the phylum NEMATODA has resulted in the fact that anything like an adequate perspective of the group as a whole has been achieved only within recent years—far later than a similar understanding of the PLATYHELMINTHES. The major groups of parasitic helminths were recognized by Zeder in 1800 and named by Rudolphi in 1808 and have largely survived as such. In this way the TREMATODA and CESTOIDEA, once established, have stood the test of time because they were from the outset essentially natural groupings of parasitic forms set off from their free-living relatives. Not so the NEMATODA (or, as Rudolphi originally called them, the NEMATOIDEA); although established as a parasitic group, it had to receive in the ensuing years free-living members. But free-living and parasitic forms were not, for over a hundred years, really integrated. This was in good measure due to the fact that study of the phylum was long carried out by two sharply separated groups—zoologists interested in the free-living nematodes and

³ I should prefer to use the more general term “symbiosis,” but refrain here because of the inevitable confusion with the common misuse of this word to mean “mutualism.”

⁴ Or three, if one accepts the very reasonable theory that the monogenetic and digenetic trematodes are of independent origin.

parasitologists interested in the parasitic ones. It would not be possible in a short symposium to go into historical detail on nematode classification. A few salient facts may, however, be mentioned to advantage. The nineteenth and early twentieth century saw no adequate system for the NEMATODA as a whole. The late N. A. Cobb attempted (1919) a classification of the nematodes treated for the first time as a phylum, but was fundamentally unsuccessful. His was based entirely, so far as one can judge from his paper, on free-living forms. Not only did he fail to integrate parasitic forms into his scheme, but as Chitwood (1950b) has explained, the cephalic characters chosen by him were not fundamental to nematode phylogeny, and consequently his groupings have not survived. His tremendous contribution to nematology, especially of free-living groups, is not thereby to be minimized, however.

Workers of this general period—for example Wülker (1924)—who treated the classification of the NEMATODA as a whole, tended to group the free-living forms along with certain clearly related parasites in a heterogeneous suborder or superfamily ANGUILLULOIDEA, parallel with a number of parasitic suborders or superfamilies. Wülker himself, however, recognized that such a group probably would have to be split into a number of coördinate groups. But, in any case, these classifications failed to provide for a real integration of parasites and free-living forms.

In 1926 Baylis and Daubney published the first monograph of the NEMATODA as a whole, in which the genera of what was to them a class were comprehensively treated. They recognized clearly the fact that “the habit of parasitism has been developed not only once, but at various times in the evolutionary history of the group”, but their efforts at fitting the free-living and parasitic groups together were of limited success because of their essential preoccupation with parasitic forms. Thus they included all the free-living genera in a single order ASCAROIDEA and ranged the parasites in this and four other orders. That Baylis and Daubney produced an essentially unnatural grouping of all free-living forms with certain highly evolved parasites in an enormous, heterogeneous order does not detract from the fact that by assembling the genera of nematodes they were responsible for a tremendous advance in the understanding of the phylum.

Filip'ev (1929) and (1934) proposed a system of classification for the NEMATODA that went a long way toward rectifying the limitations of Baylis and Daubney's scheme. His discussion (1934) of the systematic position of the NEMATODA was essentially modern—he subscribed to the ASCHELMINTHES concept. Five entirely or largely free-living orders and six parasitic orders in a class or phylum NEMATODA were recognized. Although this still largely segregated the free-living and parasitic forms in an artificial manner, he nevertheless recognized the very close affinities of the modern suborder STRONGYLINA with his order ANGUILLULATA (= modern order RHABDITIDA, in essence), in which he reduced the former to the status of a family STRONGYLIDAE, and the close relationship of the modern superfamily MERMITHOIDEA (order ENOPLIDA, suborder DORYLAIMINA), a highly specialized group of insect parasites, with his order ENOPLATA (= modern order ENOPLIDA, in essence), in which he treated the mermithoids as a family MERMITHIDAE. Moreover, he recognized the close affinities of his parasitic order OXYURATA with his largely free-living ANGUILLULATA.

It was finally the work of Chitwood (1937—see also Chitwood and Chitwood, 1937a), recently revised (1950a), that produced the first nematode classification

in which the interrelationships of the free-living and parasitic forms were essentially understood and expressed in all their complexity. To him must go credit for the first really adequate system based on phylogeny. Whatever elaborations future investigators will develop, it seems very likely that these will be on the basis of the Chitwood classification (1937, 1950a) which, with minor deviations, is used as the framework upon which the balance of this paper is organized.

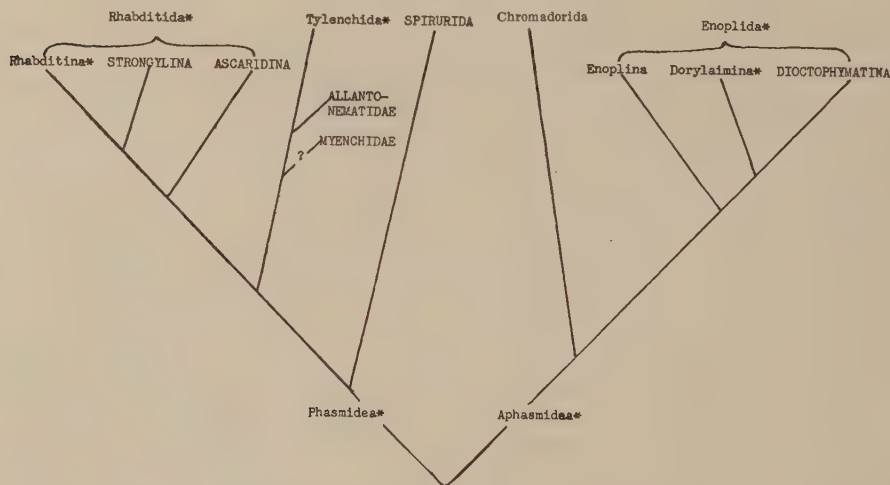


FIG. 1. The evolution of major (and two minor) zoöparasitic groups in the phylum Nematoda. (Classes end in "-idea", orders in "-ida", and suborders in "-ina". Exclusively parasitic groups are printed in capitals; partly parasitic groups are marked with an asterisk [*].)

It is worth noting here that ambitious efforts have been and are being made by Soviet helminthologists under the leadership of Skriabin to develop evolutionary theories for most of the nematode groups. Unfortunately Skriabin's example (1941, 1946) to his students and associates, (*e.g.*, Deliamure, 1950; Sobolev, 1950), who have been extending their leader's work, has been an unhappy one. Skriabin's contribution to the taxonomy of parasitic worms, extending over the past 40 years, has been enormous, but his phylogenetic speculations of the past decade are confused. Claiming to draw on evidence from various scientific disciplines, his proposed evolutionary pattern for the nematode class PHASMIDEA fails, basically because of inadequate weighing of morphological as against other evidence. Sobolev (1950) has recently attacked Chitwood for "formalism" and extolled the theories of Skriabin, which are fundamentally opposed to Chitwood's in several important respects. Although Sobolev puts forth admirably the requirements of phylogenetic reasoning when dealing with groups that lack fossil remains, the Soviet school that he represents has so far not translated these fine concepts into practice. The Soviet workers are in much need of the searching self-criticism on which they ostensibly pride themselves; it is obvious that any unfavorable commentary that I can offer will be dismissed by them on ideological grounds.

GENERAL SURVEY OF ZOÖPARASITISM IN THE NEMATODA

Twelve years ago there appeared an essay by the eminent British helminthologist, H. A. Baylis (1938), entitled "Helminths and evolution". Probably very little can be added today to his general discussion of helminth origins, about which relatively little is known. His treatment of evolution within the nematode phylum, however, was considerably limited by comparison with what can be written now. Chitwood's classification was doubtless too recent at that time for appraisal of its full implications. However, Baylis made the highly pertinent observation that "the conclusion seems inevitable that the habit of parasitism must have arisen independently in several distinct stocks [of nematodes]". In making this point he reiterated a prophetic speculation of similar nature made by Cobb as early as 1898 (pp. 453-4).

The previous year Schuurmans Stekhoven (1937b) had attempted to derive all of the major parasitic groups of nematodes except the OXYUROIDEA from the marine free-living aphasmideans. This may be dismissed, for Chitwood has assembled overwhelming morphological evidence for the phasmidean affinities of the strongylinés, ascaridoids, and spirurides. Inspection of figure 1, which is based on the Chitwood classification (1950a)—except for recognition of the order TYLENCHIDA of Thorne (1949) in the place of a suborder TYLENCHINA in the order RHABDITIDA—provides graphic representation of the complex interrelationships between free-living and zoöparasitic forms in the nematode phylum. In Chitwood's scheme there are two classes, PHASMIDEA and APHASMIDEA,⁵ of which the most primitive members are free-living. Indeed the genera *Rhabditis* Dujardin, [1844], and *Plectus* Bastian, 1865, which appear to be morphologically most primitive in their respective classes, approach one another quite closely, but from them two quite divergent groups deviate. Both classes have many parasitic members, but the PHASMIDEA demonstrate a much greater evolution of such groups.

Within the PHASMIDEA there are three orders, of which one, the SPIRURIDA, is exclusively parasitic. The other two, the RHABDITIDA and TYLENCHIDA, both include parasitic groups, particularly the former. Within the APHASMIDEA there are two orders, of which one, the CHROMADORIDA, includes, so far as known, no true parasites⁶; it is interesting to note that this order is almost exclusively aquatic and very largely marine. The other aphasmidean order, the ENOPLIDA, includes three important parasitic lines.

The greater evolution of parasitism in the PHASMIDEA seems quite clearly to be related to the fact that the free-living phasmideans are largely inhabitants of the soil, whereas the free-living aphasmideans are more characteristically aquatic and include the great bulk of marine forms. Soil-inhabitation seems peculiarly suited for the development of parasitic lines. The reason for the development of parasitism in such forms seems rather evident. There is the ever-present danger of desiccation, and a highly selective advantage would consequently reside in those organisms that could exploit their animal or plant associates in the soil as temporary refuges against such a fate. A next logical step would be the operation of evolutionary processes to stabilize such relationships, and then with the parallel evolution of host and parasite

⁵ Spelled by him PHASMIDIA and APHASMIDIA, but modified here in accordance with the uniform system of group endings suggested by Pearse (1948).

⁶ Members of the curious genus *Odontobius* Roussel de Vauzème, 1834, live as commensals on baleen plates of mysticete whales. There are a few other comparable relationships in the Chromadorida and Enoplina, especially in the gills of certain crustaceans (Chitwood, 1935).

new kinds of organisms would arise, in time abandoning much of their old morphology and assuming various and wonderful forms.

When one considers, for example, the meter-long kidney worm of carnivores, *Diectophyma renale* (Goeze, 1782) Stiles, 1901 (which, it is true, probably had an aquatic, rather than terrestrial, free-living ancestor), one can but marvel at the remarkable potentialities of evolutionary parasitism. Free-living nematodes are at most a few centimeters long! With the nematodes, parasitism seems actually to have led in many cases to elaboration of structure over that of free-living forebears, by contrast with the situation in many groups where marked degeneration has usually taken place in parasitic lines (copepods, barnacles, turbellarians, etc.).

To consider further the individual phasmidean orders, it will be noted in figure 1 that the RHABDITIDA consists of three suborders—RHABDITINA, STRONGYLINA, and ASCARIDINA, of which the last two are entirely parasitic. Within the RHABDITINA there are a number of parasitic lines that are discussed in the following section. The order TYLENCHIDA at present is not divided subordinally. It has primarily specialized in plant parasitism, but there are two animal-parasitic families, one of uncertain affinities with the true tylenchs (family MYENCHIDAE). The order SPIRURIDA, as already noted, is entirely parasitic.

As regards the aphasmidean order ENOPLIDA, there are three suborders—ENOPLINA, DORYLAIMINA, and DIOCTOPHYMATINA. Of these there are no true parasites known in the first (although some are commensalistic), but the second includes two important parasitic lines (superfamilies MERMITHOIDEA and TRICHUROIDEA), and the third is entirely parasitic.

In the past few decades descriptions have accumulated for a large number of genera and species of parasitic nematodes. A thorough survey and census of these now presents a formidable task and could scarcely have been attempted for the purpose of this symposium only. Such a study is, needless to say, of great importance and essential if we are to achieve a proper perspective over the nematodes as parasites. Yorke and Maplestone's monograph (1926) on the nematodes of vertebrates is now greatly out of date, and Baylis and Daubney's (1926) on the genera of nematodes, free-living and parasitic, equally or more so. The ensuing discussion is presented with the full knowledge that there are still wide areas of ignorance in our knowledge of the NEMATODA and that I personally am less familiar with some groups than with others. I beg indulgence for the consequent limitations of the discussion.

Two suborders have been selected here for especial scrutiny—the basically free-living RHABDITINA and the parasitic STRONGYLINA. The remaining parasitic lines are treated in somewhat less detail, but all with special reference to their host groups. In the latter connection I have been particularly aided by the reviews of Christie (1941) and Chandler, Alicata, and Chitwood (1941) on zoöparasitism in invertebrates and vertebrates respectively. These authors have approached the question of parasitism from the evolutionary point of view—more, however, with attention to the initial development of the parasitic relationship as such than to the origin of specific nematode groups or to the concomitant evolution of host and parasite—aspects with which I am particularly concerned here.

PARASITIC LINES IN THE SUBORDER RHABDITINA

The RHABDITINA are divided by Chitwood into two superfamilies—RHAB-

DITOIDEA and DRILONEMATOIDEA. The latter are curious, tiny forms, occurring in earthworms, and their specialized morphology suggests a long evolution as parasites. Their basic structure, however, appears to be rhabditine. Baylis (1943) has studied a number of drilonematoids and certain other nematodes, parasitic in earthworms, which he treats as of uncertain affinities. Chitwood (1950a) has assigned these latter to an appendix to his new superfamily DRILONEMATOIDEA. It is apparent that the nematodes living as adults in earthworms are rather diversified and as yet relatively little known. If they are a monophyletic group, they represent a very interesting evolutionary series.

The rest of the RHABDITINA belong to the superfamily RHABDITOIDEA, which comprises eight families, three of these consisting entirely of members parasitic during at least certain stages of their life cycles, and a fourth, of members that usually, although in some cases at least, not obligately, parasitize insects. The most important criteria for the recognition of rhabditoid families lie in the nature of the stoma and the esophagus.

The most primitive rhabditoids belong to the family RHABDITIDAE. *Rhabditis* is at present the largest genus of soil nematodes; it is badly in need of revision to permit the recognition of a number of genera. In any case, there are a large number of species referable to the family RHABDITIDAE and subfamily RHABDITINAE, and these present certain features that Chitwood (1950c) regards as relating them closely to the hypothetical protonematode. It is possible that forms very like the RHABDITINAE gave rise to such highly evolved parasitic groups as the suborders STRONGYLINA and ASCARIDINA. It is quite certain that *Rhabditis*-like forms gave rise directly to the rhabditine family RHABDIASIDAE.

Certain members of all four basically free-living rhabditoid families—RHABDITIDAE, CYLINDROCORPORIDAE, DIPLOGASTERIDAE, and CEPHALOBIDAE⁷—occur in intimate relationships with other animal organisms, particularly those in the soil. Species of *Rhabditis* (*sensu lato*) and other rhabditoids of free-living families have been reported in association with a wide variety of hosts—molluscs (Chitwood and Chitwood, 1934, 1937b), crustaceans (Chitwood, 1935), insects (Bovien, 1937; Filip'ev and Schuurmans Stekhoven, 1941), and vertebrates (Chitwood, 1933; Chandler, 1938).

Some of these relationships are no more than accidental, especially in the case of vertebrates as hosts. Over a decade ago Chandler (1938) reviewed literature on parasitism of vertebrates by generally free-living rhabditoids and reported a number of personally observed cases of infection in man by *Diploscapter coronata* (Cobb, 1893) Cobb, 1913. The human cases were all associated with gastric achlorhydria. Larvae of *Rhabditis strongyloides* (Schneider, 1860) Örley, 1880, have, for example, been shown to invade the skin of dogs and cause a dermatitis (Chitwood, 1932).

Other relationships appear to be, or obviously are, facultative parasitism. Many rhabditids and diplogasterids are transported as "dauerlarven" (i.e., resistant III stage larvae) by insects, to the outside of which they are attached. Some of these relationships appear rather specific (Fuchs, 1915; Bovien, 1937), even obligatory in the case of *Rhabditis coarctata* Leuckart, 1891, as described by Triffitt and Old-

⁷ Sachs's recent relegation (1950) of the CEPHALOBIDAE to the status of a subfamily in his family ANGUILLULIDAE (=RHABDITIDAE) is not in harmony with the many differences that separate the rhabditids and cephalobids. His approach to nematode classification is largely pre-Chitwoodian.

ham (1927) and verified by Bovien (1937). Other rhabditoids of the basically free-living families are known to occur as endoparasites in the gut and tissues of invertebrates. Most of these occur as larvae only (Bovien, 1937), but a few cases are reported wherein adults develop in the host—as, for example, in the case of *Diplogaster labiata* Cobb in Merrill and Ford, 1916, which parasitizes a beetle—the elm borer, *Saperda tridentata*. Even this form seems to be only facultatively parasitic inasmuch as it has apparently been found growing well on non-living substrates in nature (Merrill and Ford, 1916).

Of those hosts harboring larval stages of free-living rhabditoids, particularly studied have been earthworms and carrion beetles (Völk, 1950). Within these, certain nematode species occur with considerable regularity—most notably *Rhabditis pellio* (Schneider, 1866) Bütschli, 1873, and *R. maupasi* Seurat, 1919 (family RHABDITIDAE) in various earthworms. Johnson (1913) has especially studied the latter form. These species apparently invade their hosts as infective (most probably III stage) larvae and usually encyst therein, only developing further and completing their cycle upon the death of the infected worm. Sometimes the adult and larval nematodes are found in nature unassociated with living or dead earthworms, and they can be cultivated for years in the laboratory on nutrient agar in the presence of bacteria suitable as food⁸. This situation—a condition wherein earthworms (or other organisms) serve preferentially as “transport” hosts—seems an incipient stage in the evolution of obligate endoparasitism.

The evolutionary step from facultative to obligate parasitism has apparently been taken by a few members of the CYLINDROCORPORIDAE, DIPLOGASTERIDAE, and CEPHALOBIDAE.

Evidence for such relationships in the case of the cylindrocorporid genus *Longibucca* Chitwood, 1933 (with a snake and a bat) is to be inferred from the reports of Chitwood (1933) and McIntosh and Chitwood (1934), although this evidence is probably not definitive.

Ackert and Wadley (1921) studied the interesting case of *Cephalobium microbivorum* Cobb (family DIPLOGASTERIDAE, subfamily CEPHALOBIINAE), which they found to infect the gut of the black field-cricket, *Gryllus assimilis*.

In the CEPHALOBIDAE, *Alloionema appendiculatum* Schneider, 1859 (subfamily ALLOIONEMATINAE), presents a most fascinating picture of alternation of generations, or *heterogeny*. One or more parasitic generations occur in certain slugs alternating with one or more free-living generation (Claus, 1868). The preparasitic forms are “dauerlarven” and have to invade a slug in order to continue development. On nearing maturity the parasitic pre-adults must bore out of the host in order to copulate and reproduce. If external conditions are favorable, a free-living generation or generations are produced; if unfavorable, new “dauerlarven” result, which must reinfect a slug in order to continue development. Here one encounters a situation midway between facultative and obligate parasitism. The interesting, somewhat degenerate cephalobids of the genus *Daubaylia* Chitwood and Chitwood, 1934 (subfamily DAUBAYLIINAE), have been only found as adult parasites in the pulmonary cavity of certain gasteropod molluscs (Chitwood and Chitwood, 1934). Although definitive evidence is lacking, it seems not unlikely that the daubayliins are obligate parasites. Certain species of the genus *Cephalobus* Bastian, 1865 (sub-

⁸ I have now kept *R. pellio* in culture for over three and a half years.

family CEPHALOBINAE) have also been found as adult parasites in snails (Chitwood and Chitwood, 1937b), but the nature of the relationship is facultative.

It is interesting to note that there thus is a complete integradation from accidental to obligate parasitism in the basically free-living families of rhabditoids. Furthermore it is probably not without significance that those forms appearing to be obligately parasitic are generically or possibly even subfamiliarily (as with the DAUBAYLIINAE) distinct from their closest free-living, or at most facultatively parasitic, relatives. This is consonant with a notion that the evolution of parasitism is inevitably bound up with morphological change. It is perhaps not unreasonable to assume that the more a parasitic line deviates from its ancestral stock the longer in general have its parasitic habits existed. This point of view must, however, be carefully qualified with recognition of the fact that different lines of organisms unquestionably have different rates of evolution.

It should not come as a surprise to learn that all stages of parasitism should exist today in the basically free-living rhabditoid families. These forms are the type of evolutionary material that must provide for a constant evolutionary pressure toward parasitism.

The remaining four families of the RHABDITOIDEA are the STEINERNEMATIDAE, RHABDIASIDAE, ANGIOSTOMATIDAE, and STRONGYLOIDIDAE. Of these, the members of the first are characteristically insect parasites, but not even so much evolved in the direction of parasitism as the diplogasterid, *Cephalobium microbivorum*. Species of *Neoapectana* Steiner, 1929, have been best studied. The infective (III) larval stage of two forms, *N. bibionis* Bovien, 1937, and *N. affinis* Bovien, 1937, invade certain flies and live therein, apparently harmlessly, until the host dies, whereupon they mature and pass rapidly through several generations, finally moving out into the soil as "dauerlarven" (Bovien, 1937). *N. affinis*, if infecting a beetle rather than a fly (Bovien, 1937), and also *N. glaseri* Steiner, 1939, in all hosts so far as known (Glaser, McCoy, and Girth, 1940) grow in the living insect and usually kill it during their second generation, then passing through a number of generations in the insect cadaver. *N. bibionis* was grown on egg albumin for several generations by Bovien, and *N. glaseri* and another species, *N. chresima* Glaser, McCoy, and Girth, 1942, have even been successfully cultured under axenic conditions (i.e., in the absence of other living organisms) for numerous generations (Glaser, 1940; Glaser, McCoy, and Girth, 1942). The steinernematids thus appear to be in the nature of facultative parasites, but their relationship to insects seems quite specific in nature. Morphologically, their larvae are cephalobid-like, and they probably can be regarded as deriving from the CEPHALOBIDAE. They have however, undergone extreme degeneration of the stoma in the adult stage—some-what beyond the condition in the DAUBAYLIINAE.

The rhabdiasids and strongyloidids resemble *Alloionema appendiculatum* in exhibiting heterogeny. Both groups have members characterized by one or more parasitic generations alternating with one or more free-living generations; both are parasitic in certain vertebrates above the fishes—the rhabdiasids typically in the lungs of amphibians and reptiles and the strongyloidids in all classes. Rarely two or more generations may be passed in a single host—as in a case of human parasitism by *Strongyloides stercoralis* (Bavay, 1876) Stiles and Hassall, 1902 (see Faust, 1949, pp. 396–7).

The RHABDIASIDAE in the free-living generation are morphologically very close to the RHABDITIDAE, whereas in the parasitic they have a reduced or capsuliform stoma with thick walls. They seem to have been derived from the RHABDITIDAE as already noted. The STRONGYLOIDIDAE, on the other hand, have a reduced stoma and according to Chitwood and McIntosh (1934) could have been derived from a genus like *Alloionema* Schneider, 1859. This would place them closest to the CEPHALOBIDAE.

The angio stomatids are not a well known group. They occur as apparently obligate parasites of the gut of amphibians and snails and do not have a free-living generation so far as known. Like the strongyloids and the rhabdiasids of the parasitic stage they have undergone stomatal specialization (Chitwood, 1933). The well developed caudal alae of the male suggest rhabditid affinities.

The foregoing survey of zoöparasitic lines in the RHABDITINA has thus revealed very interesting degrees of parasitism representing all gradations from accidental through facultative to obligate relationships. The most evolved parasitic lines—RHABDIASIDAE, STRONGYLOIDIDAE, ANGIOSTOMATIDAE, and DRILONEMATOIDEA—would appear, with the exception of the last, to be somewhat more recent of origin than the group next to be considered, the suborder STRONGYLINA. They have, however, made significant advances in the evolution of parasitism. The restriction of the RHABDIASIDAE to amphibians and reptiles suggests that this family has evolved since the mammals and birds separated from the reptiles. Most likely the rhabdiasids established themselves in either the amphibians or reptiles and later spread to the other class. Antiquity of the STRONGYLOIDIDAE might be inferred from its distribution throughout the tetrapods, but in view of its relatively slight morphological deviation from the other rhabditoids, it seems more likely that it is a biologically versatile group, which established itself of fairly recent times in one vertebrate class and soon spread to the others. Similarly the ANGIOSTOMATIDAE may be relatively recent, quite possibly having established themselves first in gasteropods and then in the amphibians that fed on the latter. The DRILONEMATOIDEA, by contrast, are very likely an archaic group with a long evolution as parasites of oligochaete annelids.

From the standpoint more particularly of host distribution we have seen that the principal host groups to the rhabditines are insects, gasteropod molluscs, earthworms, and vertebrates. Of these, the first three are parasitized facultatively by many different species in basically free-living genera. In time certain of these may give rise to radiating parasitic lines. Already, of parasites in insects, there are now a number of genera in basically free-living families, and the STEINERNEMATIDAE have familial status; in molluscs, the DAUBAYLIINAE have subfamilial and the ANGIOSTOMATIDAE familial status; and in earthworms, the DRILONEMATOIDEA have superfamilial status. As parasites of tetrapod vertebrates the RHABDIASIDAE and STRONGYLOIDIDAE have achieved familial distinction.

The RHABDITINA are a veritable laboratory of evolutionary parasitism.

THE SUBORDER STRONGYLINA

Chitwood (1950a) classifies the STRONGYLINA in three superfamilies—STRONGYLOIDEA, TRICHOSTRONGYLOIDEA, and METASTRONGYLOIDEA. It seems doubtful to me that these are of value equal to the superfamilies of the suborders

RHABDITINA and ASCARIDINA. Indeed, a good case might well be made for considering the STRONGYLINA merely a superfamily STRONGYLOIDEA in the suborder RHABDITINA. The strongyline seems scarcely as different from the superfamily RHABDITOIDEA as does the superfamily DRILONEMATOIDEA. Moreover, I have felt (Dougherty, 1945, 1949) that the groups delimited by strongyline superfamilies separate families more closely related to one another than to some of those placed in the same superfamily. Some of the reasons for this view have already been presented in a recent paper (Dougherty, 1949) although my arguments therein are somewhat weakened by what I now regard as an erroneous interpretation of the structure and affinities of certain strongyline lungworms (Dougherty, 1951). Further comparative study of the fundamental structure of the strongyline is needed. For the purposes of the present survey I recognize six families, without superfamilial groupings—SYNGAMIDAE, DIAPHANOCEPHALIDAE, STRONGYLIDAE, METASTRONGYLIDAE, ANCYLOSTOMATIDAE, and TRICHOSTRONGYLIDAE. Of these, the SYNGAMIDAE and DIAPHANOCEPHALIDAE appear to be most primitive.

By comparison with the parasitic families of the RHABDITOIDEA the suborder STRONGYLINA, which is almost certainly a monophyletic line, seems quite clearly of considerable antiquity. The strongyline is distributed quite widely in all vertebrate classes above the fishes and are specialized in such a way that a considerable period of concomitant host-parasite evolution seems evident. On the basis of comparative morphology and embryology it is apparent that the STRONGYLINA arose from rhabditoids similar to the RHABDITIDAE. The more primitive strongyline recapitulate the development of the rhabditid stoma and esophagus almost perfectly in their early larval stages. So far as I have been able to determine, this was first noticed as regards the esophagus by Leuckart (see 1868, p. 436) and has been commented upon by many subsequent workers, in greatest detail by Looss (1911).

Inasmuch as the free-living rhabditoids are primarily inhabitants of the soil, it is reasonable to believe that parasitizing of the vertebrates by the earliest strongyline did not begin until after the transition from an aquatic to a partially terrestrial existence was effected by the vertebrates. Thus it could easily have been that the AMPHIBIA were first to serve as hosts to the incipient strongyline group. With the semi-aquatic existence and frequently moistened skins of amphibians in mind, it is easily conceived how the stem rhabditoids may have developed a burrowing pattern into the drying skin of early amphibians to avoid desiccation and thus have become adapted to an inner environment by migration through the blood stream to the lungs and later to the gastrointestinal tract. Such a burrowing pattern is exemplified by a number of the rhabditoid groups already considered. An initial phase is well illustrated by the burrowing tendency of *Rhabditis strongyloides* as already described. More advanced are the rhabdiasids and strongyloidids, which typically penetrate the skin of their hosts, migrating to the lungs via the circulatory system. In both cases and continuing on to the gastrointestinal tract in the latter.

It would thus seem likely that skin-penetration was the primary mode of entry of the primitive strongyline. This view is also subscribed to by Chandler, Alicata, and Chitwood (1941). In present-day strongyline it is characteristic of the ancylostomatids as a group, with a few exceptions, and of certain trichostongyline and syngamids. For reasons to be discussed directly one cannot expect to find

a modern-day representative of the early strongyline, and thus there probably can be no direct proof of the primitiveness of skin-penetration.

Chitwood (1950c) has briefly discussed strongyline evolution, and I have sketched my own conception of it in abstract (Dougherty, 1946). Skriabin (1941) has propounded an elaborate theory of phasmeidan evolution in which certain specialized metastrongylids are considered primitive and account not only for the origin of the other STRONGYLINA, but for the order SPIRURIDA and suborder ASCARIDINA! His system is exceedingly artificial and without basic logic; I have criticized it elsewhere (Dougherty, 1944, 1949). The most important point to be considered in a critique of strongyline evolution is that the majority of host groups which these parasites must have exploited in the course of their history have perished. Today we have only a remnant of the classes AMPHIBIA and REPTILIA and are not a great deal better off with the class MAMMALIA. Despite this fact, a critical survey of the STRONGYLINA and correlation of their probable evolution with that known for their hosts are not without reward.

The AMPHIBIA were a large and important assemblage in the Carboniferous age, between 300 and 200 million years ago; thereafter they declined and perished except for three relict groups—the urodeles, anurans, and blind-worms (caecilians). Of these, the urodeles may even be of origin separate from that of the other amphibians and higher tetrapods. If the amphibian class included, as suggested, the initial hosts to the developing strongyline suborder, these early parasites might easily have failed to survive in the specialized modern members of the group. In fact, the modern amphibians have no convincingly primitive strongyline parasites; such as they do have belong only to the TRICHOSTRONGYLIDAE, a specialized family most characteristic of mammals (Travassos, 1937).

In considering the REPTILIA as hosts of the STRONGYLINA, one is faced with a problem very similar to that of the AMPHIBIA. The reptiles radiated particularly during the Mesozoic, from 200 to about 60 million years ago. Thereafter they declined, although less extensively than the AMPHIBIA. It is perhaps possible that the strongyline arose as parasites of the reptilian class. I believe that adequate evidence exists that they could not have arisen with any higher group of vertebrates. The family DIAPHANOCEPHALIDAE is restricted to the reptilian order SQUAMATA (snakes and lizards) and is thus a group peculiar to reptiles. It has an unusual specialization of the stoma to form two lateral jaw-like parts, but the cephalic papillary pattern is as primitive as that of any strongyline group and more primitive than most. Chitwood and Wehr (1934), as is discussed in the next section, have considered cephalic papillary patterns of prime importance in working out aspects of evolution in the order SPIRURIDA, and it seems to me that they are of evolutionary significance in the STRONGYLINA as well. Strongyline genera other than those of the DIAPHANOCEPHALIDAE found in the REPTILIA (e.g., *Sauricola* Chapin, 1924, *Oswaldocruzia* Travassos, 1917, etc.) appear to be closely related to genera in the MAMMALIA and more reasonably represent secondarily acquired parasites from the latter class than primary parasites of reptiles. Nevertheless, the DIAPHANOCEPHALIDAE may be the only surviving one of many strongyline families otherwise characteristic of, but now extinct along with, the great orders of Mesozoic reptiles.

The family SYNGAMIDAE might also have had its origin in the REPTILIA inas-

much as its members are rather primitive (*i.e.*, more nearly rhabditoid) in their cephalic, labial, and papillary pattern; however they are today found only in birds and mammals. The subglobular, transversely hexagonal stoma of the SYNGAMIDAE can probably be regarded as slightly less specialized than the bivalvular stoma of the DIAPHANOCEPHALIDAE. It may therefore be that the syngamids represent the few survivors of a large primitive group of strongyline. This seems probable from a consideration of them in relation to their hosts. *Cyathostoma* Blanchard, 1849, and *Syngamus* von Siebold, 1836, are found in the respiratory tract of birds, and the latter genus also in that of mammals; but because *Cyathostoma* is the less specialized genus, it seems probable that species of *Syngamus* were secondarily acquired by mammalian from avian hosts. *Deletrocephalus* Diesing, 1851, is found in the intestinal tract of a bird—*Rhea americana* (order RHEIFORMES); this is a less specialized location than that of *Cyathostoma* and *Syngamus* and gives strength to the concept that birds are more primitive hosts of the SYNGAMIDAE than are mammals. *Stephanurus* Diesing, 1839, is an aberrant genus occurring in the kidney and perirenal tissues of the pig (order ARTIODACTYLA). The other genus that I might place in the SYNGAMIDAE—namely *Acheilostoma* Leiper, 1911—is not well known; it seems most closely to resemble *Deletrocephalus* and is found in mammals (orders RODENTIA and PERISSODACTYLA) in the gall bladder or intestine. Possibly the SYNGAMIDAE arose with the stem reptiles from which both mammals and birds later evolved, or perhaps they originated in the reptilian group (order THECODONTIA) from which the birds arose, or in the birds themselves, and later became established in mammals as well. At any rate here is further evidence that the strongyline may have originated at least with stem reptiles.

By far the greatest number of strongyline species—most of the STRONGYLIDAE and TRICHOSTRONGYLIDAE, and all of the ANCYLOSTOMATIDAE and METASTRONGYLIDAE—occur in the class MAMMALIA. The modern flowering of the suborder has occurred in this group of hosts. It is of further interest that there is remarkable correlation between the dietary habits of the mammalian groups and the diversity of their strongyline parasites. Thus it is in the herbivorous orders that the most extensive radiation of strongyline types has taken place. The STRONGYLIDAE are best represented in the herbivorous MARSUPIALIA and in the PERISSODACTYLA and PROBOSCIDEA. The TRICHOSTRONGYLIDAE are most developed in the XENARTHRA, RODENTIA, and ARTIODACTYLA. The ANCYLOSTOMATIDAE and METASTRONGYLIDAE are somewhat less strikingly radiated in herbivores, although in both families there is evidence of particular success in herbivorous groups.

The family STRONGYLIDAE as I have delimited it (Dougherty, 1946) is a large group that on basic comparative study may prove divisible into two or more families. I am not, however, satisfied with the efforts so far made (Ershov, 1943; Chitwood, 1950a). Chitwood has recognized a family CLOACINIDAE, but in the absence of a comprehensive review of the strongylid genera (*sensu lato*), it is not clear that this family can be set off distinctly from the family STRONGYLIDAE as Chitwood defines it. Characteristically members of the broad strongylid group have a fusion of the eight submedian cephalic papillae of the external circle into four, often setose, duplex papillae. I would recognize three strongylid subfamilies—CLOACININAE, OESOPHAGOSTOMINAE, and STRONGYLINAE. I thereby divide the subfamily CYATHOSTOMINAE (= TRICHONEMINAE, or TRICHONEMATINAE) of many authors between the CLOACININAE and OESOPHAGOSTOMINAE.

It seems obvious that the most primitive of the STRONGYLINA, after the diaphanocephalids and syngamids, belong to the subfamily CLOACININAE, for the cloacinins characteristically have six well developed labia and relatively primitive stomata, although in reduction of cephalic papillae they are now more specialized than the diaphanocephalids and syngamids. The cloacinins are best represented and appear to have radiated most in the Australian herbivorous diprotodonts (order MARSUPIALIA) as the extensive work of Johnston and Mawson (1940) has shown. Australia has been isolated since Cretaceous times, and it thus seems an unavoidable conclusion that the ancestors of the cloacinins were present at that time. The occurrence of cloacinin genera—*Kiluluma* Skriabin, 1916, etc.—among placentals (orders PERISSODACTYLA, PROBOSCIDEA) suggests further that these primitive strongylids were present in the ancestors of both the marsupial and placental mammals—which were seemingly the pantotheres (order PANTOTHERIA) of the Jurassic. The strongylid family itself may have originated in this group or possibly even in the ancestral mammal-like reptiles (order THERAPSIDA). Characteristic of the STRONGYLIDAE is the internal corona radiata, which either is primitively lacking or has been secondarily lost by certain modern cloacinin genera, *e.g.*, *Zoniolaimus* Cobb, 1898, as redefined by Kung (1948).

The two other strongylid subfamilies—OESOPHAGOSTOMINAE and STRONGYLINAE—have seemingly departed more from a primitive cephalic organization than the modern CLOACININAE, for their lips have undergone a subdivision into the external corona radiata. The OESOPHAGOSTOMINAE in stomatal characters appear closer to the CLOACININAE, hence somewhat more primitive than the STRONGYLINAE. Both subfamilies probably evolved after the separation of the marsupials and placentals inasmuch as members of neither occur in the MARSUPIALIA of Australia.⁹ It is impossible to be sure of the mammalian group to which they are normal. Possibly they have evolved with the PERISSODACTYLA and become secondarily acquired by other mammalian orders, especially the PROBOSCIDEA, although they may have originated with the common ancestors of both groups.

Perhaps the origin of the STRONGYLIDAE is more ancient than I tend to believe, however. There is at least one strongylid genus peculiar to birds (*i.e.*, *Codiostomum* Railliet and Henry, 1911) and one to reptiles (*i.e.*, *Sauricola* Chapin, 1924), the former falling in the STRONGYLINAE and the latter in the OESOPHAGOSTOMINAE. The host in the first case is an ostrich; in the second, a turtle. Now both of these vertebrates are among the most primitive surviving members of their respective classes, and it may well be significant, from the phylogenetic viewpoint, that they harbor strongylids. However, against the view that they are primary hosts to the STRONGYLIDAE may be raised the already mentioned points, first, that both *Codiostomum* and *Sauricola* represent advanced strongylid types and, second, that these types are not represented, so far as known, in Australian marsupials. It therefore seems more likely that strongylids were transferred to birds and reptiles from their primary habitation in mammals.

The evolutionary relationship of the STRONGYLIDAE to the other three strongyline families not yet considered is largely in the realm of conjecture. However, if one

⁹ Johnston and Mawson (1940) place *Globocephaloides* Yorke and Maplestone, 1926, and *Oesophagostomoides* Schwartz, 1928, in the STRONGYLINAE; I should place the former genus in the ANCYLOSTOMATIDAE (subfamily GLOBOCEPHALINAE) and the latter in the CLOACININAE.

considers cephalic characters, one finds that the STRONGYLIDAE and METASTRONGYLIDAE on one hand basically have six lips—or a derivative thereof in the form of the corona radiata of the oesophagostomins and strongylins—and that on the other hand the TRICHOSTRONGYLIDAE largely and the ANCYLOSTOMATIDAE entirely lack lips. Because of these structural conditions, I believe that there occurred, at least by the time of the pantotheres and possibly earlier, a split of the strongyline of mammals into two main stalks, one leading to the STRONGYLIDAE and METASTRONGYLIDAE, and the other to the TRICHOSTRONGYLIDAE and ANCYLOSTOMATIDAE.

The family METASTRONGYLIDAE includes the most specialized members of the STRONGYLINA. All metastrongylids are polymyarian, and the stoma has become vestigial, *i.e.*, they are *meiostomatous* (Dougherty, 1945). In all forms the bursa of the male is somewhat reduced in size, and in some this has proceeded so far that bursal rays are entirely lacking as such. Typically the female genital tract has undergone a striking subtotal fusion of the ejectors of the more typical strongyline ovejectoral apparatus into a long cylindrical vestibulum; this is, however, not true of a group of genera with amphidelphic uteri. The metastrongylids are all parasites of the respiratory or circulatory system of mammals. Nevertheless, despite their specializations, these strongyline possesses one character more primitive than the corresponding one in the STRONGYLIDAE—namely that the dorsal and ventral papillae of the external circle of cephalic papillae are not fused into four submedian papillae, but are separate in the METASTRONGYLIDAE. The relict metastrongylid genus *Heterostrongylus* Travassos, 1925, has been described from a South American opossum. The METASTRONGYLIDAE may therefore have originated before the marsupials and placentals split. However, they have never been reported from Australian marsupials. In any event, it would seem very likely that the lung-inhabiting METASTRONGYLIDAE arose from an early, gut-inhabiting strongylid, similar to, but in some respects (*e.g.*, in labial characters) more primitive than, the modern CLOACININAE.

I have discussed at length elsewhere the interesting radiation of the METASTRONGYLIDAE (Dougherty, 1949; 1951). It is possible to follow one line, the subfamily PROTOSTRONGYLINAE, that has evolved basically in the ARTIODACTYLA, another, the PSEUDALIINAE, in the odontocete CETACEA, and two others, the FILAROIDINAE and SKRJABINGYLINAE, in the CARNIVORA. In addition the subfamily METASTRONGYLINAE contains the apparently relict genus *Metastrongylus* Molin, 1861, in pigs and tentatively the genus *Heterostrongylus* Travassos, 1925, in an opossum. The genus *Dictyocaulus* Railliet and Henry, 1907 (subfamily DICTYOCAULINAE), principally in the ARTIODACTYLA, is of uncertain affinities, in some respects being rather trichostrongylid-like.

Some years ago I referred the curious genus *Metathelazia* Skinker, 1931, to the company of certain other metastrongylids of carnivores in the subfamily FILAROIDINAE (Dougherty, 1943). Recently Gerichter (1948) has described some new species in this genus which demonstrate striking variability in cephalic characters. This fact throws doubt on my placement of *Metathelazia* and indicates that the genus as constituted at present actually consists of more than one genus, which may represent an independent group of some antiquity. I am now disposed to separate them out as an independent subfamily, VOGELOIDINAE subfam. nov., which is being treated in detail in a separate paper (Dougherty, in press). For a diagnosis of this subfamily see the appendix to this section.

The fact that both the METASTRONGYLIDAE and the TRICHOSTRONGYLIDAE are primarily meiostomatous might suggest that these families are closely related. Indeed it might seem attractive to derive the former from the latter family through a form like *Dictyocaulus*. This derivation encounters a fundamental obstacle in the nature of cephalic characters. *Metastrongylus*, an apparently primitive genus, has six massive labia or lips, whereas *Dictyocaulus* has none. Other metastrongylids have six distinct, although in most cases less well developed labia¹⁰ than those of *Metastrongylus*. Even the presumably most primitive genera of the TRICHOSTRONGYLIDAE, i.e., those with the best developed stomata (*Amidostomum* Railliet and Henry, 1909), have only slightly developed lips, and the vast majority of trichostrongylids appear to lack labial structures. It therefore seems quite unlikely that *Dictyocaulus* represents a transitional form between the metastrongylids and trichostrongylids. *Dictyocaulus* may indeed represent an aberrant trichostrongylid genus that has evolved convergently with the metastrongylids in adopting a pulmonary habitat. In cephalic characters it is more trichostrongylid than metastrongylid (cf. Chitwood, 1950b, fig. 56S); and like the trichostrongylids, but unlike the definite metastrongylids, it has a direct life cycle.

The ANCYLOSTOMATIDAE and TRICHOSTRONGYLIDAE are morphologically closely related. They are somewhat more specialized than the STRONGYLIDAE in that in both families there are no well developed lips—in the ANCYLOSTOMATIDAE none at all in fact—although in most the cephalic papillae are less reduced than in the STRONGYLIDAE. Furthermore, the great reduction of the stoma in most trichostrongylids and the peculiar development of ventral cutting plates or of ventral biting teeth called *odontia* in most ancylostomatids are rather striking specializations.

Ancylostomatids and trichostrongylids are found in Australian marsupials, and this is strong evidence that the families arose before the marsupials and placentals split. Members of the ANCYLOSTOMATIDAE are today found only in mammals, whereas those of the TRICHOSTRONGYLIDAE occur in all tetrapod classes. In fact among the living strongyline only trichostrongylids occur in the AMPHIBIA. However, two of the three genera whose species parasitize amphibians are also represented in reptiles; these are, according to Travassos (1937), *Oswaldocruzia* Travassos, 1916, and *Amphibiophilus* Skriabin, 1916. The third genus, *Schulzia* Travassos, 1937, is peculiar to the AMPHIBIA, whereas the genera *Herpetostrongylus* Baylis, 1931, and *Trichoskrjabinia* Travassos, 1937, are peculiar to the REPTILIA. In the AVES there are also certain genera peculiar to the class: *Amidostomum* Railliet and Henry, 1909, *Ornithostrongylus* Travassos, 1914, etc., although *Libyostrongylus* Lane, 1923, is found in both birds and mammals. It is perhaps true that most of these genera exhibit obvious, though small, stomata, with teeth arising in the posterior part of the small stoma and termed *onchia*; there is even some degree of stomatal sclerotization—especially in *Amphibiophilus* and *Amidostomum*. However, these structures of the trichostrongylid genera occurring in hosts outside the class MAMMALIA are not necessarily more primitive than those of certain trichostrongylids occurring in mammals. There seems no evidence to suggest that the TRICHOSTRONGYLIDAE arose with the AMPHIBIA and evolved along with the higher vertebrate classes. Rather, as I have suggested, it appears that both the TRICHO-

¹⁰ Gerichter (1949) calls them "perityls", but I see no particular advantage in this special term.

STRONGYLIDAE and ANCYLOSTOMATIDAE arose with the early MAMMALIA or possibly the mammal-like reptiles (order THERAPSIDA); the former family appears to have been peculiarly adaptable and spread secondarily to members of the other tetrapod classes, while the latter has proved more conservative and less successful.

In the ANCYLOSTOMATIDAE I should recognize three subfamilies—GLOBOCEPHALINAE, UNCINARIINAE, and ANCYLOSTOMATINAE. The first of these subfamilies apparently represents the most primitive of the surviving ancylostomatids. Although the globocephalins in common with other ancylostomatids have no lips or mouth collar and like almost all ancylostomatids possess an anterodorsally flexed oral opening, they have not evolved either ventral odontia or ventral cutting plates. Members of this subfamily occur in marsupials, primates, and artiodactyls. The subfamily UNCINARIINAE is represented in Australian marsupials (the genus *Hypodontus* Mönnig, 1932) and in placental orders and presumably arose from a globocephalin stem before the separation of the marsupials and placentals. *Hypodontus* is interesting in that it is the only ancylostomatid with an anteroventrally flexed oral opening (Mönnig, 1929). The ANCYLOSTOMATINAE are, so far as known, restricted to the placentals. The uncinariins and ancylostomatins as groups demonstrate no striking host preference.

Two of the three subfamilies of the TRICHOSTRONGYLIDAE on the other hand show a rather interesting host-restriction, but not the TRICHOSTRONGYLINAE, which is best considered a rather broad group including those forms closest to the stem trichostrongylids. In point of number of genera and species the TRICHOSTRONGYLIDAE constitute the most successful of present-day strongyline families. From the standpoint of host-relationships they appear to be the most adaptable of the suborder and have undergone conspicuous morphological radiation, although the less numerous METASTRONGYLIDAE are rivals in this respect. Aside from the TRICHOSTRONGYLINAE the two other subfamilies are rather obviously of recent origin and show a degree of host-preference which gives excellent evidence of their ancestral host groups. Essentially they are rather small homogeneous offshoots presenting anatomical features that nicely fit subfamilial categories. In am opposed, therefore, to the degree of subdivision championed by Travassos (1937), whose classification of the TRICHOSTRONGYLIDAE into thirteen subfamilies is not, I believe, based on characters of evolutionary significance. I should, for the time being at least, recognize only subfamilies TRICHOSTRONGYLINAE, STRONGYLACANTHINAE, and HELIGMOSOMINAE. The first subfamily includes the genera in members of the non-mammalian classes; it also includes, among others in placentals, most of the gastrointestinal meiostomes in ruminants, which constitute a considerable number of genera, and also some of those in marsupials and all in monotremes. The subfamily STRONGYLACANTHINAE has apparently evolved with the bats (order CHIROPTERA); the genus *Bradypostrongylus* Price, 1928, probably has become secondarily adapted to sloths (order XENARTHRA). The HELIGMOSOMINAE probably began to differentiate at least with the ancestral insectivores from which the XENARTHRA and RODENTIA have originated; the most primitive genera—e.g., *Trichohelix* Ortlepp, 1922, which has two complete ovaries, and *Moennigia* Travassos, 1935, in which the posterior ovary is atrophied, but part of the uterus and the posterior ovejector remain—occur in armadilloes (order XENARTHRA), and the more specialized genera, with at most a vestige of the posterior ovejector, occur in both xenarthrans and rodents. The

heligmosomins of the modern insectivores may have evolved with their hosts, or have been derived from one of the other orders—probably the rodents.

From the foregoing account, certain generalizations can be made by way of summary. The STRONGYLINA have by no means preserved strict host-specificity, but enough of the ancestral pattern appears to have survived to make possible certain reasonable conclusions of a general nature on the pattern of their evolution. Most strikingly the DIAPHANOCEPHALIDAE, being specialized and at the same time restricted to snakes and lizards, suggest an antiquity of the suborder at least as great as that of the early reptiles. The family SYNGAMIDAE, being represented in both birds and mammals, with the more primitive members in the former, is probably the most primitive surviving strongyline group. Finally the families STRONGYLIDAE, METASTRONGYLIDAE, ANCYLOSTOMATIDAE, and TRICHOSTRONGYLIDAE appear to have arisen with the early mammals or the mammal-like reptiles. Evidence for interesting evolutionary patterns of concomitant parasite-host evolution is preserved in the METASTRONGYLIDAE and TRICHOSTRONGYLIDAE, especially in the former.

APPENDIX

Since the foregoing discussion makes use of a classification proposed originally in abstract (Dougherty, 1946) and slightly modified here, but never explained in detail, an outline classification of the STRONGYLINA is appended at this point:

SUBORDER STRONGYLINA Pearse, 1936. Eustomatous or meiostomatous; labia 6 or 0, sometimes subdivided into an external corona radiata; esophagus more or less clavate in adult stage; intestinal cells polynucleate; terminal excretory duct tubular; excretory canals H-shaped with two subventral glands; meromyarian or polymyarian; male: bursa with rays typically present, but sometimes secondarily lacking—when present, peloderan (with rare exceptions); female: reproductive system highly developed, uteri terminating in ovejectors, each consisting typically of infundibulum and ejector and joining with its fellow at the vagina. (In adult stage, parasites of vertebrates.)

FAMILY DIAPHANOCEPHALIDAE Travassos, 1920. Stoma in form of two lateral jaws; lips and corona radiata absent; submedian cephalic papillae of external circle not fused; meromyarian. (Parasites of gut of snakes and lizards; monoxenous.)

FAMILY SYNGAMIDAE Leiper, 1912. Stoma subglobular, hexagonal in cross section; oral opening hexangular; 6 labia vestigial; submedian cephalic papillae of external circle not fused; meromyarian. (Parasites of gut of birds, respiratory tract of birds and mammals, and kidney or gall bladder of mammals; monoxenous.)

FAMILY STRONGYLIDAE Baird, 1853. Stoma globoid, cylindroid, or infundibuliform, never hexagonal in cross section; jaws absent; 6 labia present or divided into external corona radiata: internal corona radiata usually present; submedian cephalic papillae of external circle fused into four conoid, often setose papillae; meromyarian. (Parasites of gut of vertebrates, characteristically mammals; monoxenous.)

Subfamily Cloacininae Stossich, 1899. Stoma short and cylindroid or infundibuliform; dorsal onchium absent; six distinct,

sometimes massive labia characteristically present. (Parasites of marsupials and placentals.)

Subfamily Oesophagostominae Railliet, 1915. Stoma long or short and cylindroid, infundibuliform, or rarely subglobular, with opening of dorsal esophageal gland at tip of short onchium, never extending almost to stomatal margin; labia divided into external corona radiata. (Parasites characteristically of placentals.)

Subfamily Strongylinae Railliet, 1885. Stoma globular or subglobular with opening of dorsal esophageal gland at tip of massive onchium extending almost to stomatal margin; labia divided into external corona radiata. (Parasites characteristically of placentals.)

FAMILY METASTRONGYLIDAE Leiper, [1909]. Meistomatous without onchia; 6 labia usually present; submedian cephalic papillae of external circle not fused; polymyarian. (Parasites of respiratory or circulatory system of mammals; heteroxenous so far as known, except Dictyocaulinae.)

Subfamily Metastrongylinae Railliet and Henry, 1909. 6 well developed labia; male: bursa small; rays misshapen and irregularly reduced in size; gubernaculum simple or lacking; female: prodelphic; vestibulum without terminal muscularization; provagina present or absent. (Parasites of pigs and opossums; intermediate hosts earthworms where known.)

Subfamily Filaroidinae Skriabin, 1933. 6 poorly developed labia; gubernaculum sometimes showing clearly developed, although small capitulum, otherwise simple or even lacking; female: vestibulum without terminal muscularization; provagina absent. (Parasites most characteristically of carnivores; intermediate hosts gasteropods where known.)

Subfamily Vogeloidinae, subfam. nov. 6 well or poorly developed labia, or lacking; inner circle of cephalic papillae lacking; male: bursa lacking, rays papillary and strung along either side of anus in non-strongyline pattern; female: provagina absent; vestibulum without terminal muscularization, but vulva with valve-like structure. (Parasites characteristically of carnivores; intermediate hosts unknown.)

Subfamily Skrjabinogylinae Skriabin, 1933. 6 poorly developed labia, or lacking; male: bursa and rays usually of good size (exception: *Skrjabinogylus*); gubernaculum simple or absent; female: amphidelphic. (Parasites characteristically of carnivores; intermediate hosts gasteropods.)

Subfamily Pseudaliinae Railliet and Henry, 1909. 6 poorly developed labia; male: bursa small or lacking; rays fused into five trunks with these variously reduced; female: vestibulum with special sphincter vestibuli distally; provagina present or

absent. (Parasites of odontocete cetaceans; intermediate hosts unknown.)

Subfamily Protostrongylinae Kamenskii, 1905. 6 poorly developed labia; male: bursa well developed or imperfect; rays rarely misshapen (except dorsal ray); female: prodelphic; vestibulum without terminal muscularization; provagina often present. (Parasites of ruminants and occasionally lagomorphs; intermediate hosts gasteropods.)

Insertae sedis: Subfamily Dictyocaulinae Skriabin, 1933. Large worms; labia lacking; male: bursa large with long rays; female amphidelphic. (Parasites characteristically of ruminants; monoxenous.)

FAMILY ANCYLOSTOMATIDAE Nicoll, 1927 (Syn. Agchylostomidae Looss, 1905; Ancylostomidae Lane, 1917). Stoma subglobular, not hexagonal in cross section; oral opening unguarded, or guarded by odontia or cutting edges; labia and corona radiata absent; submedian cephalic papillae of external circle not fused; meromyarian; in female, proximal end of ejector modified to form sphincter. (Parasites of gut of mammals; monoxenous.)

Subfamily Globocephalinae Travassos & Vogelsang, 1932. Oral opening unguarded by odontia or cutting edges.

Subfamily Uncinariinae Stiles, 1903. Oral opening guarded by cutting edges.

Subfamily Ancylostomatinae Nicoll, 1927 (Syn. Agchylostominae Looss, 1905; Ancylostominae Stephens, 1916). Oral opening guarded by odontia.

FAMILY TRICHOSTRONGYLIDAE Leiper, 1912. Meiostomatous, often with an onchium; 6 vestigial or 0 labia; submedian pairs of cephalic papillae of external circle fused or not fused; meromyarian or polymyarian; ovejectoral apparatus similar to that in the Ancylostomatidae. (Parasites of gut of vertebrates, most characteristically mammals; monoxenous.)

Subfamily Trichostrongylinae Leiper, [1909]. Vulva equatorial or slightly pre- or postequatorial, or rarely at posterior end in front of the anus; ovejectoral apparatus always removed some distance from posterior end; two ovaries; no caudal mucrones in female.

Subfamily Strongylacanthinae Yorke & Maplestone, 1926. Vulva always approximately equatorial; two ovaries; caudal mucrones present at female posterior end.

Subfamily Heligmosominae Travassos, 1914. Vulva close to, or immediately in front of, anus; ovejectoral apparatus at posterior end; one or rarely two ovaries; caudal mucrones absent.

OTHER PARASITIC LINES IN THE NEMATODA

I do not propose to consider the remaining parasitic nematode lines in the same detail as those of the RHABDITINA or as the STRONGYLINA. The treatment of the latter can serve as a frame of reference for further consideration of concomitant

vertebrate-nematode evolution, for phylogenetic analysis of major parasitic lines involves similar considerations. The general problems of speculation on parasitic nematode evolution should be evident from the foregoing discussions and will, I hope, lead other investigators to approach in detail the remaining nematode parasitic lines from the standpoint of reconstructing probable patterns of host-parasite evolution. Chitwood (1950c) has briefly considered these lines.

The ASCARIDINA are almost certainly a more archaic group than the STRONGYLINA. Chitwood recognizes superfamilies OXYUROIDEA and ASCARIDOIDEA. Of these, some of the oxyuroids are relatively little differentiated from some of the rhabditoids. In fact, the steinernematids were originally placed in the OXYUROIDEA. Members of the primitive oxyuroid family THELASTOMATIDAE occur in the gut of arthropods, particularly insects, and members of the somewhat more specialized family RHIGONEMATIDAE are peculiar to the gut of millipedes. The remaining two families, OXYURIDAE and ATRACTIDAE, are most characteristic of vertebrates, although one whole attractid subfamily, the RANSOMNEMATINAE, is restricted to arthropods.

The ASCARIDOIDEA are largely parasites of vertebrates, although a few members of the primitive family COSMOCERCIDAE are also parasites of the gut of gasteropod molluscs. Chitwood feels that the ascaridoids are derived from the oxyuroids, but no modern representative of the latter qualifies as a member of the ancestral group inasmuch as all existing oxyuroids have lost the ventrolateral cephalic papillae and the cervical papillae, or *deirids*, all of which the ascaridoids have retained.

In contrast to the STRONGYLINA it seems logical to assume that the ASCARIDINA began as parasites of arthropods, perhaps with some stem mandibulate group. It is important to note that in the Silurian there are fossil millipedes, which are terrestrial mandibulate arthropods, whereas the first known amphibians were late Devonian, at least 50 million years later in time. Inasmuch as terrestriality is much more ancient in the arthropods than in the tetrapods, it would not have been surprising to find a broad radiation of ascaridines in the mandibulate ARTHROPODA—particularly the insects. That this is not the case is perhaps related to the fact that in their evolution most of the higher insects developed life patterns to which the ascaridines could not adjust. Thus we find oxyuroids today in the cockroaches, which are primitive insects, and in scarabaeid beetles, which, though more highly evolved, have retained habits easily compatible with infection by soil dwelling nematode larvae. Contrast this situation with the higher vertebrates, which were fated to take their strongylinae along with them in their evolution and provided for extensive radiation of these parasites in relatively recent times. Oxyuroids of millipedes and insects have probably changed but little over hundreds of millions of years, in keeping with the relatively slight evolution of their hosts. Here we have in striking contrast two host groups with markedly different rates of evolution, and their parasites of immediate interest to us demonstrate a parallelism of rate with their hosts.

The oxyuroids most probably became secondarily adapted to vertebrates from ancestors in arthropods. Inasmuch as no ascaridoids are known to occur in contemporary arthropods, their origin may have been from a primitive oxyuroid stock in vertebrates. For the most part today the vertebrate hosts of the ascaridines are tetrapods, and it seems logical to assume that the transfer of oxyuroids to vertebrates

occurred after the evolution of the AMPHIBIA. In their probable time of becoming vertebrate parasites the ascaridines thus resemble the strongylinas. There are oxyuroids in fish, but these are of scattered occurrence. A few ascaridoids also occur in fish, but these belong largely to the highly specialized family ASCARIDIDAE (subfamily ANISAKINAE) and are related to forms in aquatic tetrapods. Here, indeed, is a strikingly clear example of the transfer of a parasitic line between two quite different host groups.

The order TYLENCHIDA has primarily specialized in plant parasitism. However, the remarkable family ALLANTONEMATIDAE consists of insect-parasites. As Christie (1941) has remarked, it is probable that only a small proportion of the existing species have been described. From the basic pattern of the life cycle, which is reviewed in detail by Christie, it is apparent that the allantonematids have succeeded where the oxyuroids have failed—namely the former have been able to adapt to the various life habits of the higher insects and occur in all groups. It would be fascinating to attempt a correlation of insect and allantonematid evolution; I have not done this by reason of my relative unfamiliarity with the family. The family MYENCHIDAE appears to be related to the TYLENCHIDA, but is imperfectly known; Chitwood (1950a) places it as an appendix to the "TYLENCHINA." Its members occur in leeches and amphibians (Pereira, 1931).

The SPIRURIDA, an entirely parasitic order, is possibly the most archaic such line in the NEMATODA. It has presumably lost the morphological features that could suggest affinities to free-living forms, for except in basic phasmeidan features similarities between its members and the rhabditoids are minimal. Chitwood suggests that the free-living ancestor might have been like the modern CYLINDROCORPORIDAE. As adults the spirurides are all found in vertebrates, and an unusual feature of the entire order is that parasitism of an intermediate host, usually an arthropod, is universal. Where intermediate hosts are required in other nematode groups, this need does not characterize a larger systematic unit than a family (*e.g.*, the METASTRONGYLIDAE).

Chitwood recognizes two spiruride suborders—CAMALLANINA and SPIRURINA, based essentially on the nature of the larval phasids. Representatives of both suborders are found in all vertebrate groups including the fish. This might suggest an origin of the order as parasites of fish, possibly before the development of the tetrapods. However, there are few aquatic, free-living phasmeidans, and it seems more likely that the SPIRURIDA first inhabited the land vertebrates and spread to the fishes later. It is possible to postulate that the need for an intermediate host was established very early and that all surviving spirurides have retained the pattern, or that arthropods were the original hosts and that, when the spirurides were transferred to vertebrates, either arthropods were retained as intermediate hosts, or then were reacquired as hosts, but only to the intermediate stages of the parasites. The latter possibilities are entirely speculative, but could provide for a much greater antiquity of the SPIRURIDA than if they were primary parasites of vertebrates. There is the difficulty that no adult spiruride at present parasitizes arthropods or other invertebrates, but this problem might be answered if the ancestral host group were to have perished.

Schuermans Stekhoven (1937a) suggested the origin of the SPIRURIDA from the ENOPLIDA, basing his theory essentially on supposed similarities in cephalic papilla-

tion. This scheme, however, ignores the basically phasmeidan excretory system and the presence of phasmids and is quite untenable. Chitwood and Wehr (1934) exhaustively studied the cephalic structure of the camallanine superfamily CAMALLANOIDEA and the spirurine superfamily SPIRUROIDEA and worked out what they considered the most probable evolutionary interrelationships. This has never been extended to the rest of the order—superfamilies DRACUNCULOIDEA (CAMALLANINA) and FILARIOIDEA (SPIRURINA). I shall not attempt here a further correlation of host and parasite evolution in the SPIRURIDA, although such would probably prove quite rewarding.

The three aphasmidian lines of parasitic nematodes are either dorylaimine (superfamilies MERMITHOIDEA and TRICHUROIDEA) or closely related to the suborder DORYLAIMINA (suborder DIOCTOPHYMATINA). The mermithoids are curious creatures, their larval stages parasitizing arthropods, both aquatic and terrestrial while the adults are free-living. The trichuroids on the other hand are parasites of vertebrates of all classes, and the most primitive genus is probably *Cystoopsis* Wagner, 1867, which occurs in fish. Quite probably both groups were of aquatic origin.

The suborder DIOCTOPHYMATINA is a small group occurring as adults only in birds and mammals. Recently Woodhead (1950) has described the first stage larva of *Dioctophyma* Collet-Meygret, 1802, which clearly possesses a dorylaimoid stomatal stylet and esophagus. He also described what he claims to be second stage larvae, and these are apparently indistinguishable from certain larval stages of the phylum NEMATOMORPHA. Chitwood (1950c) has expressed doubt that these are actually larval *Dioctophyma*, although, as described by Woodhead, the experiments in which they were obtained were conducted with great care. It is, however, true that larval nematomorphs were recognized by Woodhead in the same intermediate hosts (certain leeches) as his supposed larvae of *Dioctophyma*. It is essentially impossible to accept any real relationship between the DIOCTOPHYMATINA and the NEMATOMORPHA, for the latter have a number of fundamental structural features that remove them from any intimate relationship with the NEMATODA. If Woodhead's observations are accurate, a remarkable convergence is evident between larval dioctophymatines and gordiaceans.

It seems clear that the DIOCTOPHYMATINA are close to, even though much modified from, the DORYLAIMINA.

SUMMARY

I have in the foregoing discussion considered in some detail the basically free-living suborder RHABDITINA and the parasitic suborder STRONGYLINA and somewhat superficially five other major and two minor parasitic nematode lines. The rhabditines present a fascinating picture of parasitic evolution in its inception and in various stages of early success—even possibly ancient success in the case of the drilonematoids; the strongylinae, a picture of successful host-parasite evolution with considerable radiation over vast ages.

It is obvious that nematodes are at once basically very successful as free-living forms and yet remarkably adaptable to parasitic life. In the latter connection it has been the nematodes of the soil that have succeeded most. Probably all groups of land animals and plants have become victims of these versatile parasites, and in the case of some animal host groups it is, as we have seen, possible to reconstruct something of the evolution of certain parasitic lines.

A new classification of the STRONGYLINA is presented in outline. A new sub-family, Vogeloidinae (family Metastrongylidae), is proposed.

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ON THE LIFE CYCLE OF *TRYPANOSOMA CRUZI**

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The life cycle of *T. cruzi*, as judged from textbooks and other literature of the last 40 years, seems well established. The trypanosome, we are told, for its multiplication becomes a leishmanian round form and develops through a leptomonad and crithidial stage again into trypanosomes, this being the identical evolution in all known media: the invertebrate host, the vertebrate host and in artificial cultures. While the evolution from leishmania to crithidia presents essentially a growing up process into an elongated and mature flagellate, easy to be followed under the microscope, the transformation of the crithidia into trypanosome is said to occur by a diminution in size and a migration of the kinetoplast from its prenuclear position to the posterior pole of the cell: "Les Crithidia diminuent de taille, leur blépharoplaste émigre et on obtient finalement de vrais trypanosomes" (Brumpt, 1912).

This theory, although since then generally recognized (Craig, 1948), was not accepted by Carlos Chagas and has been rejected by the writer (1940, 1942, 1943, 1944) who, in addition, presented another experimentally supported concept of the development of the trypanosome, *according to which the trypanosome develops, in every medium, directly from round forms*. The fact that these findings remained unnoticed, unquoted or completely misunderstood (Hoare, 1945) and that experimental work performed during recent years brought important evidence in favor of the author's views, is the reason for his coming back to the subject.

The idea that the trypanosome form develops from crithidia is based on the fact that stained smears of *Triatoma* feces and cultures may contain parasitic cell forms with sufficient intermediate stages of kinetoplast position to suggest a successive transformation of crithidia into the trypanosome form. But if that should be the case, there is no explanation for the fact that many cultures which contain abundant trypanosomes and crithidias, lack these intermediate stages. On the other side, when these intermediate stages do exist, another explanation is at hand which is positively based on direct observation. Such forms appear in the course of the retrogressive transformation of *T. cruzi* into the leishmanian round form, a process which has been described briefly by C. Chagas (1909) and in detail by the writer (1942, b) and was later confirmed by Muniz and de Freitas (1946). It takes place, within a few hours, when trypanosome-containing blood is held at room temperature, sowed into culture media, sucked by *Triatoma* or inoculated into the vertebrate. But it also occurs in cultures in which new trypanosomes have been formed, as was shown by the writer (1944), and is according to his observations obviously a general habit which the trypanosome form and other evolutionary stages adopt in unfavorable conditions in order to protect themselves and to preserve the species, a concept with which Muniz and Borriello (1945) agree. During that process a true migration of the kinetoplast takes place, but in a sense opposite to that supposed in the theory of the transformation of crithidia into trypanosome, namely, from the retronuclear to the prenuclear position. Therefore, the assumption was made that

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the intermediate stages of kinetoplast position found in the smears had to be explained in that way and not according to the presently accepted theory.

A direct observation which possibly was considered as supporting Brumpt's theory was made by Fantham (1911), according to which this author followed under the microscope the transformation of a round form ("latent body") of *T. rhodesiense* into a trypanosome and observed during this process a stage that "somewhat resembled a Crithidia." However, it is obvious that this process can not be compared with the assumed transformation of the crithidia into trypanosome in *T. cruzi*. In Fantham's observation the whole development from the latent body to the trypanosome did not take more time than one hour, there was not a decreasing, but an *increasing* of size from the crithidia-like stage to the trypanosome, and the process was observed in a species (*T. rhodesiense*) where trypanosomes with posterior position of the nucleus are frequent and specific. Thus, the process differs completely from the supposed transformation of crithidia into trypanosome in *T. cruzi*.

Furthermore, Brumpt's theory of the development of the trypanosome form from the crithidia gives no plausible reason for the strange *process of rejuvenescence* which had to be assumed together with the theory; since in Brumpt's developmental schemes (1936, figs. 147 & 152) the crithidias which develop into trypanosomes are big and broad mature cells with a highly differentiated protoplasm, a round compact nucleus and a rodlike kinetoplast, while the trypanosome is a very slender, flexible form with undifferentiated protoplasm, an elongated, light, spongy nucleus and a round kinetoplast.

An important objection against the current theory is also the *striking scarcity of crithidia in the organs of the vertebrate*, while round forms and trypanosomes (these especially in artificial infections) can easily be detected. If, as that theory assumes, the crithidia is a necessary link between leishmania and trypanosome and the direct pre-stage of the latter, it should be found at least in the mixed leishmania-trypanosome accumulations and with similar frequency as the trypanosome. But this is not the case, and this situation can best be explained by the supposition *that the crithidia stage does not develop in the vertebrate*. There exist, in fact, a few references to crithidia in vertebrates in the literature, but it was shown (Elkeles, 1944) that all of them are insufficiently proved, in no reasonable proportion to the trypanosome findings, and may be explained as retrogressive stages from the trypanosomic to the leishmanian form. Thus the assumption seemed justified (Elkeles, 1940) *that the trypanosome developed here directly from round forms without intervention of the crithidia* which so regularly develops in common cultures and in *Triatoma*.

The possibility of such an evolution of the trypanosome in the vertebrate, at least in principle, has already been recognized by Brumpt (1936, fig. 147), Mayer and da Rocha-Lima (1914), Wenyon (1926, fig. 207), and today a number of new facts, experimentally proved in the last few years, tend strongly to support this view.

Muniz and de Freitas (1946), in an attempt to study the development of the trypanosome in artificial conditions which resembled as closely as possible those existing in the vertebrate, were able to confirm unequivocally that the trypanosomes developed directly from round forms, without any intervention of the crithidia. Interestingly enough, crithidias appeared in their cultures too, but only long after the trypanosomes, namely, these in 3 to 4 days, the crithidias not before 8 days,

"when the cultures became old." This is easily explained by the supposition that in their aging cultures the conditions in the medium become more and more like those existing in common artificial cultures.

In connection with these ideas, must also be cited the discovery of Muniz and Borriello (1945) that normal serum has a selectively destructive effect on crithidias while leishmanias and trypanosomes are not affected at all. Accordingly, this observation gives a plausible explanation for the fact that crithidia, in current conditions, are unable to develop in the vertebrate.

On account of the fundamentals presented, the assumption seems justified that *the trypanosome develops in the vertebrate from round forms without intervention of the crithidia*. But, as mentioned before, it is the author's opinion that this is *not a special condition of the vertebrate, but a general rule valid for all media where the trypanosome form develops*. The observations which lead to this concept, and which are given in the following paragraphs, will seem less strange if it is kept in mind that (1) it is definitely demonstrated that the trypanosome form *can* develop directly from round forms and (2) the trypanosome apparently *does not* develop from the crithidia.

In artificial cultures where leishmanian, leptomonad and crithidial forms have developed in a clear succession, the appearance of the trypanosome is a somewhat surprising and puzzling feature. Chagas was the first to wonder where they came from and which were their pre-stages. The author, in his attempt to clear this problem (Elkeles, 1942a) observed that trypanosomes were not developing everywhere in the cultures, but did so inside the sediments consisting of blood cells, detritus and parasites; here they seemed to have their "hatching places" and from here they eventually entered the condensation liquid. That this behavior remained unknown, may be due to the fact that the material for preparations is generally taken from the fluid which gives morphologically much more satisfactory results in the staining than material taken from the clumps. Nevertheless, the study of these places is of special interest, for they represent real "centers of production" where the trypanosomes can be found "as in pure culture." This recalls the situation in the vertebrate, the lumps being compared with the tissues and the fluid with the blood stream.

It seems interesting in that connection that Kofoid, Wood & Mc Neil (1935), who studied the development of the trypanosome in tissue cultures, reported: "Trypanosomes of the type usually found in the circulating blood . . . were at first localized as though emerging from the massed leishmaniaform phases." Also an observation of Muniz and de Freitas (1946) may be cited as confirming this concept. As was mentioned, these authors cultured *T. cruzi* in peritoneal fluid of guinea pigs; the fluid was obtained by intraperitoneal injection of glucose broth and contained different types of cells. Cell-free, blood trypanosomes sowed into this medium easily developed, after adopting the leishmania form, into trypanosomes, but did so only in the presence of these peritoneal cells, never without them; their existence was, as the authors stated, a condition "sine qua non." Since no multiplication inside the peritoneal cells was observed, it seems that in these cultures the sedimented cells played the same rôle for the production of trypanosomes as the "hatching places" in common cultures.

The observation that the cell clumps were the "hatching places" of the trypano-

somes lead to the supposition that also their pre-stages might be found there. In fact, this was the case; they existed in the form of minute round bodies composed of larger or smaller pairs of nuclei, frequently without visible cytoplasm, like diplococci, and together with them all the intermediate stages between these "elementary pairs of nuclei" and the perfect trypanosomes. The true size of the minute round forms and the morphology of the intermediate stages is hard to describe because of the above mentioned difficulties of their quick fixation inside the clumps and a successful staining; also the trypanosomes are in these parts much smaller and structurally less differentiated than those of the fluid. Nevertheless, the observation permitted the assumption that also in common cultures the trypanosomes develop directly from round forms.

The next question was the source of the minute round forms. Also to that question the examination of the "hatching places" of the trypanosomes gave an answer: they develop here as filial cells from big crithidias which have undergone a process of multiple division of the nuclei and can be seen amidst the masses of trypanosomes and their pre-stages. One of the cultures studied (the conditions of which, by the way, could not be duplicated) gave such excellent results in the staining of the smears that this whole process of evolution could be followed with all the desired completeness of structural detail (Elkeles 1940, 1942). The round forms, the intermediate stages and the trypanosomes of this culture were morphologically identical to those found by Mayer and da Rocha-Lima (1914) in the vertebrate, to those observed by the author in the feces of *Triatoma* (see below), and also by Muniz and de Freitas (1946) in cultures made in peritoneal exudate of guinea pigs. Consequently, *it was concluded that this type of evolution of the trypanosome is a common feature in all known media.*

The just mentioned process of multiple division of crithidias was not the only one observed in cultures of *T. cruzi*. The author (1942) found a similar process of multiple division in leishmanias, and here the process seemed to be simultaneous with, and related to, the production of very small leishmanias which were named "micro-leishmanias" and which, eventually, continued the process of simple binary fission and also of multiple division of their nuclei. This observation seems to be a parallel to that made by Mayer and da Rocha-Lima (1914) in the vertebrate, where these authors found two types of leishmanias clearly different in size (big and small ones) and structure, and considered the "small leishmanias," because of their much greater structural likeness with the trypanosomes, as the intermediate stage between the "big leishmanias" and the trypanosomes. Thus, the idea seems not beyond reason that the "microleishmanias" are identical with Mayer and da Rocha-Lima's "small leishmanias" and direct pre-stages of the trypanosome form.

The author was able to detect a type of development identical with that seen in the vertebrate and in cultures also in the invertebrate host. When the feces of infected *Triatoma* were studied with the help of a special technique of wet fixation and the elimination of the urates, the above mentioned "leishmanias of 2nd order," "elementary pairs of nuclei" and all their intermediate stages to the trypanosome were found (Elkeles, 1940), so that also in this medium the evolution of the trypanosome from the round form was clearly suggested.

According to the exposed facts and reasonings, the author concludes that the leishmanian round form has two alternative ways of evolution, namely one toward

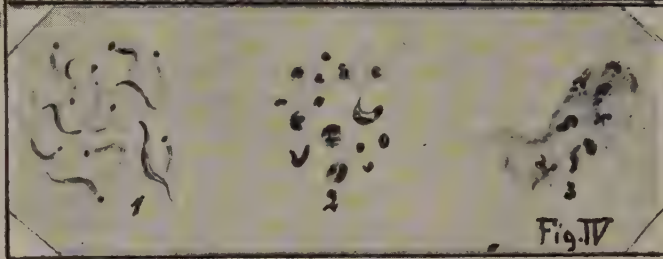
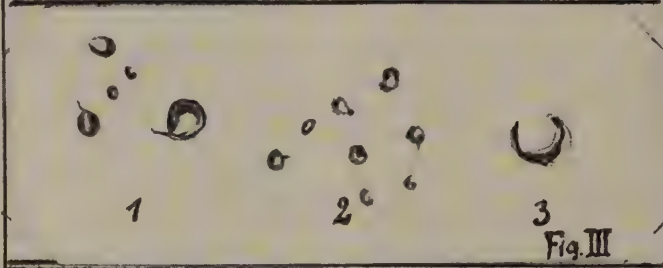
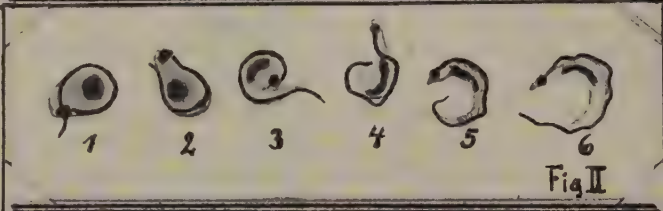
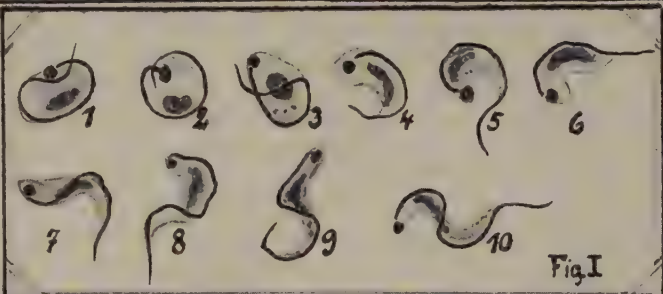
the crithidia and the other toward the trypanosome. Which one of these two features takes place, depends on the history of the round form and the conditions existing in the medium. It is not supposed that any leishmania may develop indiscriminately into either of these two forms. If that were the case—accepting the non-transformation of crithidia into trypanosome—there would be no explanation of that fact that in common cultures trypanosomes appear regularly with a delay and after crithidias have developed; likewise, in that case also in the vertebrate a quicker and more constant re-transformation of the tissue leishmanias into trypanosomes could be expected. Instead it is believed that the leishmanias which in cultures and in *Triatoma* initiate the multiplicative process, and their filial generations, develop into crithidias, while the trypanosomes are derived from other round forms produced, secondarily, through the effect of special influences or evolutive processes. As influences of that type can be considered, in a general sense, moderately unfavorable circumstances which obstruct and retard in certain degree the successive binary fission of the leishmanian, leptomonad and crithidial forms. Under these circumstances multiple divisions and probably other still unknown mechanisms take place which give rise to round forms of the type of “leishmanias of 2nd order,” “micro-leishmanias” and “elementary nuclei-pairs” which have been found to be the pre-stages of the trypanosomes and to grow and transform into them.

In common cultures, the conditions favoring the development of trypanosomes are supposed to exist preferently inside the bigger clumps of sedimented erythrocytes, parasites and detritus, because it is here where the trypanosomes are found first “as in pure culture” together with their pre-stages. Inside these masses there might be a shortage of oxygen or food which retards the uninterrupted binary fission and favors the conditions for the formation of the type of round forms which develop into trypanosomes. The observation made by Meyer (1949), in the work with Romaña and de Oliveira, may be quoted here for confirmation that the “Leishmania form in the cell protoplasm (of the tissue culture cell) divides by binary division until the cell is completely filled and only then the transformation into the flagellated form begins.”

In the vertebrate, the inter- or intracellular position of the parasites and the normal and specific defence mechanisms of the host could provide the conditions which, according to the same principle, favor the development of trypanosomes. With even more reason the necessary conditions can be supposed inside the digestive tract of *Triatoma*, especially the Malpighian tubes, and the tissues where leishmania accumulations have been found (de Faria and Cruz, 1927).

There is no sufficient support for considering multiple division processes as indispensable for the development of trypanosomes, because cells with multiple division of the nuclei often are not seen in cultures, *Triatoma* feces or vertebrate tissues which contain trypanosomes. It is therefore supposed that there exist still other mechanisms which influence leishmanian forms to develop into trypanosomes or give rise to the other round pre-stages of the trypanosome. That might explain the fact that so consistently two different types of trypanosomes are found by all observers in the development of *T. cruzi*. It may be remembered that even binary longitudinal division of *T. cruzi* has been observed (Elkeles, 1942) and that modes of behavior have been seen by various investigators (Adie, 1921, in *leishmania*, Muniz, 1927 and Elkeles, 1944 in *T. cruzi*) which suggest the existence of sexual processes. Al-

PLATE I



though these types of evolution may represent rather potential than actual faculties of *T. cruzi* in its development, we do well to admit the possibility of different mechanisms in the evolution of the trypanosome form and not to consider the life cycle of *T. cruzi* as a closed chapter.

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EXPLANATION OF PLATE

Evolution of *Trypanosoma cruzi* Chagas, 1909.

FIG. I. Evolution of the trypanosome from the round form. 1 to 3 "Leishmanias of 2nd order"; 4 to 10 "un-rolling" of the round form into the trypanosome, as found in one special artificial culture.

FIG. II. The same, in feces of *Triatoma*.

FIG. III. Stages of trypanosome development in feces of *Triatoma*; 1 to 3 sectors of the same microscopic field.

FIG. IV. Stages of trypanosome development as found in the sediments of common artificial cultures. 1 trypanosomes "as in pure culture"; 2 "elementary nuclei pairs", intermediate stages and finished trypanosomes; 3 origin of the intermediate stages from big cells showing multiple division of the nuclei.

FIG. V. Round forms found in aging artificial cultures: a, aging leishmanias; b, "micro-leishmanias"; c, aging leishmanias with multiple division of nuclei.

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EFFECT OF SIZE OF INFECTIVE DOSE, PARTIAL ILEECTOMY,
AND TIME ON INTENSITY OF EXPERIMENTAL INFEC-
TIONS OF *EUHAPLORCHIS CALIFORNIENSIS*
MARTIN, 1950 (TREMATODA)
IN THE CAT

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INTRODUCTION

Many authors, Africa (1938a, b), Africa and Garcia (1935), Africa, Leon and Garcia (1936a, b), Balozet and Callot (1939), Martin (1950a, b), Stunkard and Willey (1929), Willey and Stunkard (1942), Witenberg (1929), etc., have demonstrated that heterophyid trematodes can develop in a variety of definitive hosts. However, little attention has been paid to the relationship between the number of metacercariae fed and the number of adults recovered. An exception is Garcia's (1936) study on the effects of different methods of preparing fish for human consumption, commonly employed in the Philippines, upon metacercarial survival. But, he dealt with very small numbers for in no case were more than thirty metacercariae fed to one animal.

It has been shown by Africa (1937, 1938b), Africa, Garcia and Leon (1935), Africa, Leon and Garcia (1935, 1936a, b, c, d, 1937, 1940) that the intestinal wall of man and a few other animals may be invaded by heterophyids which may then die and release their eggs into the vascular system with serious results. Little is known, however, concerning the manner of entrance of the eggs into the blood vessels.

It was hoped that the present research might throw some light upon these problems if the cat could serve as a definitive host for the heterophyid, *Euhaplorchis californiensis* Martin, 1950, and was susceptible to invasion. As the work progressed and it was found that *E. californiensis* could develop to maturity in the cat, especially in the lower ileum and large intestine, an attempt was made to determine the effect of ileectomy and duration of the infection upon the intensity of infection.

MATERIALS AND METHODS

The cats used in the experiments were obtained as young kittens and were kept under laboratory conditions until they were at least six months of age before they were fed fish tissues infected with the metacercariae of *Euhaplorchis californiensis*. Martin (1950a) has shown that the metacercariae of *E. californiensis* encyst upon the brains of *Fundulus parvipinnis parvipinnis* (Figs. 3 and 4). Therefore attempts were made to infect cats by feeding only the heads of naturally infected fishes collected at Venice, California, but the cats refused to eat them. However, when the fish heads were mixed with commercial cat food they were readily taken. Commercial cat food was used as a basic diet for experimental and control cats.

In Series I the experimental infections were purely qualitative to determine whether or not *E. californiensis* could develop in cats. Each of three cats was fed

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several dozen infected fish heads once per week for three consecutive weeks. No attempt was made to determine the number of metacercariae fed. Cat 1 was examined at three weeks, cat 2 at four weeks, and cat 3 at five weeks after the beginning of the experiment. Control cat 4 was fed only commercial cat food.

Although the results of these experiments will be considered in detail later, infections of *E. californiensis* were established in large numbers in the lower portions of the ilea of all experimental cats. The question arose as to what would be the effect of surgical removal of that portion of the ileum in which concentrations of worms normally occur upon the intensity of infection. Therefore, under ether anesthesia, the lower four inches of the ileum of cat 5 were removed leaving the ileocolic sphincter intact. After the cat had recovered from the operation (about two weeks) it was fed infected fish heads which resulted in the establishment of an infection of low intensity in the ileum.

The results of these preliminary experiments indicated the need for more quantitative data, so a second series of infections was conducted with attention given to the number of metacercariae fed each cat and to the number of adults recovered from various areas of the intestine.

A large number of *Fundulus* was collected at Playa del Rey, California, and the number of metacercariae per fish for ten fishes was determined. The average number per fish was 253 metacercariae.

Four cats (6, 7, 8 and 9) were used in the second series of experiments. Each cat was fed twenty-five *Fundulus* heads per day for three consecutive days. On the basis of an average of 253 metacercariae per fish, each cat received approximately 18,975 metacercariae. Two weeks prior to the experimental feedings, cat 6 had the lower four inches of its ileum removed and cat 7 had the posterior eight inches of its ileum removed. Cats 8 and 9 were not operated upon but were used as controls.

Cats 6, 7 and 8 were sacrificed and examined ten days after the initial feeding. Cat 9 was killed three weeks after the initial feeding. The small intestine, cecum, colon and rectum were removed from each cat, cut into segments approximately two inches long, and the number of worms in each segment and the total for each cat determined.

Portions of the ileum from cats 1, 2, 3, 6 and 7 were paraffin embedded, sectioned, and stained with Heidenhein's modification of Mallory's azan technique.

A third series of experiments was conducted to determine the duration of the infection after a single infective feeding. Each of four cats, (10, 11, 12 and 13), was fed ten heavily infected *Fundulus* which were obtained from Newport Bay, California. A conservative estimate, based upon partial counts, indicated that there were at least 2,000 metacercariae per fish so that each cat received approximately 20,000 metacercariae.

RESULTS

The data obtained from the first series of experiments revealed that cats could serve as hosts for *Euhaplorchis californiensis* and that the control cat was negative. In all infections the worms were found in large numbers in the last six to eight inches of the ileum and in the colon. The infection of cat 5, the first cat to be infected following the removal of the posterior four inches of its ileum, resulted in a low intensity infection in the ileum.

Microscopical examination of serially cut sections of the lower ileum failed to reveal any indications of invasion or tissue destruction. Examination of the heart, lungs, liver, pancreas, spleen and blood vessels failed to reveal worms, worm fragments, or eggs. However, the adult worms frequently work their way quite deeply into the crypts between the villi (Figs. 1 and 2).

The results of the second series of experiments involving cats 6, 7, 8 and 9 are as follows. Ten days after the initial feeding, 831 adult *Euhaplorchis californiensis* were recovered from the colon of cat 6 (from which the posterior four inches of the ileum had been removed) but only fifteen worms were found anterior to the ileo-colic sphincter. Ten days after the initial feeding, 683 worms were recovered from the colon of cat 7 (from which eight inches of the ileum had been removed) but only twenty-three worms were found in the ileum. Control cat 8 was not operated upon, and was also sacrificed ten days after the initial feeding of infected fish heads. Two thousand five hundred eleven *Euhaplorchis californiensis* were recovered from the intestine, 661 from the ileum and 1850 from the colon.

Cat 9 was killed three weeks after it received the first feeding of infected *Fundulus* heads and 573 adult *Euhaplorchis californiensis* were found in the intestinal tract, sixty-six from the ileum and 507 from the colon. This reduction in number of worms recovered suggested that the infections might be self limiting.

No tissue invasion was found in the microscopic examinations of ileum sections of the second series of cats.

In the third series of experiments involving cats 10, 11, 12 and 13, cat 10 was killed and examined eight weeks after the infective feeding and adult worms were found in the lower two inches of the ileum and in the large intestine. Cat 11, killed and examined nine weeks after infection, also had adult worms in the lower ileum but none in the colon. However, cats 12 and 13, killed and examined after eleven and twelve weeks respectively, had no parasites.

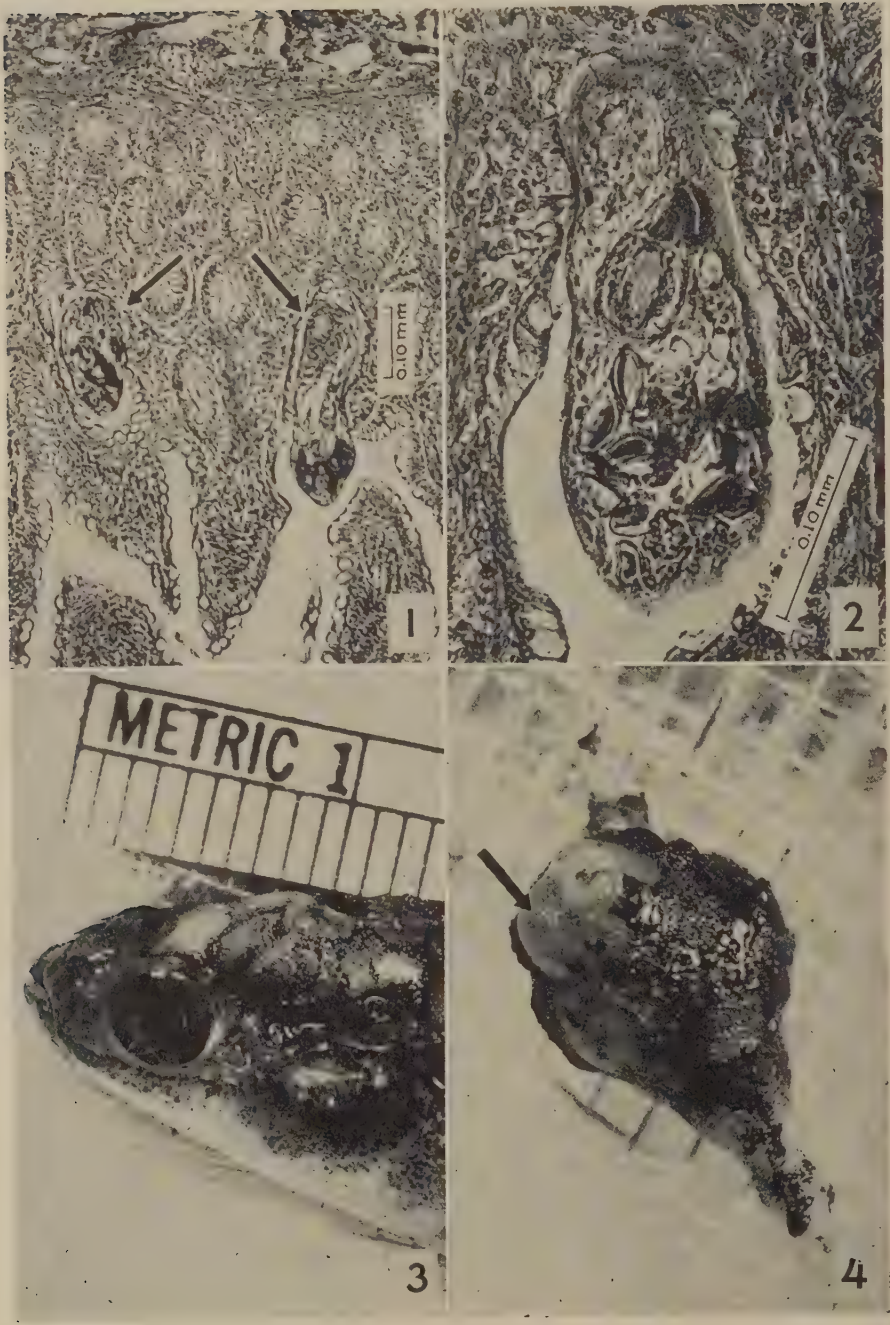
DISCUSSION

The removal of four or eight inches of ileum seems to markedly decrease the number of parasites recovered from the remaining ileum, fifteen worms from cat 6 and twenty-three worms from cat 7 as compared to 661 recovered from control cat 8. This operative procedure also may reduce the number of worms in the colon but not to the same degree as in the ileum. Eight hundred thirty-one and 683 worms were recovered respectively from the colons of cats 6 and 7, while 1850 worms were recovered from the colon of control cat 8. Thirteen per cent of the 18,975 metacercariae fed to cat 8 were recovered from the intestine as adults.

Cat 9 represents an exploratory experiment to determine the effect of a greater lapse of time on the intensity of the infection, and the decrease in the number of parasites recovered from this cat suggests the possibility that the infections might be self limiting. The results of the third series of experiments, involving cats 10, 11, 12 and 13, seem to indicate that such is the case.

The absence of tissue invasion is in agreement with results of Stunkard and Willey (1929) and Willey and Stunkard (1942) obtained with another heterophyid, *Cryptocotyle lingua* (Creplin).

PLATE I



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EXPLANATION OF PLATE

FIGS. 1 and 2. Sections of cat ileum showing adult *E. californiensis* in crypts between villi.

FIG. 3. Head of *Fundulus parvipinnis parvipinnis* (Girard) with brain exposed to show encysted metacercariae of *E. californiensis*.

FIG. 4. Same brain shown in Fig. 3 removed from skull (arrow points to some of the metacercariae).

TETRATHYRIDIDUM SP. IN A SYKES' MONKEY (*CERCOPITHECUS
ALBIGULARIS*) FROM GIZA ZOOLOGICAL GARDENS, EGYPT

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On November 2, 1950 the authors had the opportunity to examine a Sykes' monkey, *Cercopithecus albigularis* for post-mortem findings. This monkey was obtained from Kenya Colony, Central Africa in April 1950 and had been kept at the Giza Zoological Gardens ever since. Two weeks before its death, the animal developed paresis of the hind limbs, followed by paraplegia ten days later.

Upon examination, a milky white slimy clump of cestode-like ribbons was found free in the pelvic cavity around the urinary bladder. (Fig. 1.) The serous membranes in the vicinity showed petechial haemorrhages and a round inflamed area about 2 cm. in diameter was noticed on the wall of the urinary bladder.

The material was collected and examined at once in the living condition. Some specimens were pressed, fixed and stained and the remainder were preserved in 70% alcohol without pressure.

Examination of the living material revealed that it was composed of cestode-like ribbons which were actively elongating and contracting at different parts of their bodies, exhibiting pseudosegmentation and great variation in length and breadth. Each, however, did not exceed 90 mm. in length and 2 mm. in maximum breadth in the fully relaxed stage. (Fig. 2.)

Microscopical examination of the stained and pressed specimens disclosed that the bodies of these helminths are symmetrical and covered with a thin plain cuticle with a ruffled like edge. The parenchyma shows a dense thin outer layer and a structureless inner layer, both heavily studded with highly refractile "calcareous corpuscles." Longitudinal muscle fibres run throughout the whole body and are most visible in the constricted parts.

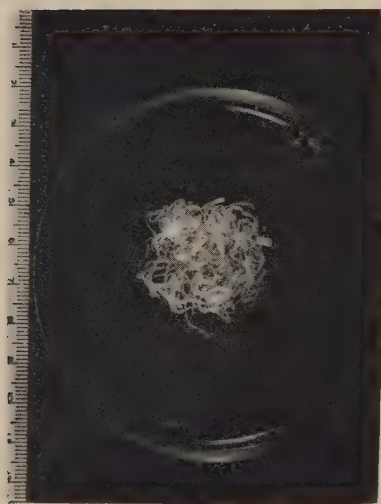
The anterior part has a completely invaginated scolex with four well developed suckers and an anterior muscular mass similar to an unarmed rostellum. The suckers are slightly oval ($104 \times 130 \mu$ approximately), with slit-like openings and devoid of any hooks or spines. (Fig. 3.)

The posterior end is blunt with a notch in the middle. (Fig. 4.)

The excretory system is composed of two main tortuous lateral canals joined by collateral branches of complicated distribution. The two main excretory canals unite at the posterior end and open into the posterior notch. In some pressed specimens the excretory canals were not visible.

DISCUSSION

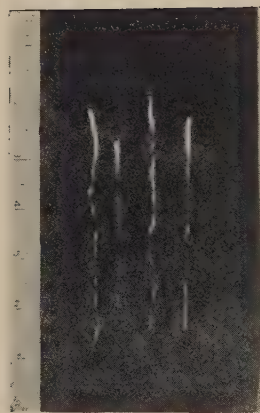
From the description of the material, both living and fixed specimens, the helminths are identified as *Tetrathyridium* Rudolphi, 1819 (larval stage of *Mesocestoides*). It is impossible to give an accurate diagnosis of the species of *Tetrathyridium* without recovering the mature *Mesocestoides* after experimental feeding of live tetrathyridia to susceptible definitive hosts. Feeding experiments could not



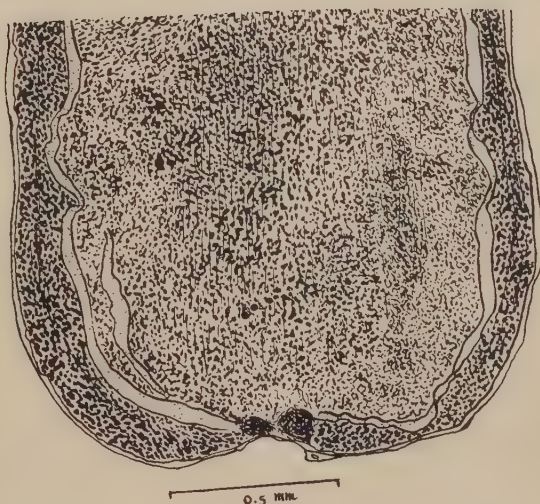
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3



2



4

EXPLANATION OF PLATE

FIG. 1. *Tetrathyridia* as recovered from the pelvic cavity.

FIG. 2. Specimens of *Tetrathyridium* sp.

FIG. 3. Anterior end of *Tetrathyridium* sp. showing the invaginated scolex.

FIG. 4. Posterior end showing the main excretory canals.

be undertaken in this case as the authors had no access to *Mesocestoides*-free dogs or cats at the time these tetrathyridia were collected.

Tetrathyridium has been recorded from several species of reptiles, birds and mammals. Though it has been recorded before from monkeys, yet as far as the authors could gather from available literature, this is the first record from "*Cercopithecus albigularis*." Moreover, this seems to be the first case of *Tetrathyridium* in Egypt. Whether the animal contracted this infection in this country or not, cannot be ascertained. *Mesocestoides* has a cosmopolitan distribution and *M. elongatus* Meggitt, 1928 has been recorded from a wolf in Egypt.

The presence of *Tetrathyridium* in monkeys raises the possibility of infection of other primates, including man. Sambon (1907), DeMeillon and Leech (1943) and others reported cases of sparganosis in human tissues from African natives. Referring to their descriptions of the specimens involved, it was noticed that no definite description of the scolex is given. *Tetrathyridium* can be mistaken for *Sparganum* unless the formation of the head is verified.

SUMMARY

1. *Tetrathyridium* sp. (larval form of *Mesocestoides*) is recorded from the pelvic cavity of a Sykes' monkey, *Cercopithecus albigularis*, for the first time in Egypt.
2. Description of the larval cestode is given, including the invaginated head.
3. Attention is drawn to the importance of describing the heads in identifying and recording the larval cestodes.

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DEVELOPMENT OF THE MOTHER SPORO CYST AND REDIAE OF *PARAGONIMUS KELLICOTTI* WARD, 1908

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INTRODUCTION

Ameel (1934) worked out the life cycle of *Paragonimus kellicotti* in experimental infections of *Pomatiopsis lapidaria*. Later, Chen (1937) made a study of the germ cell cycle of this species in which she followed the germinal cells in sections through all the stages of development. This was the most complete study of its type that had been made up to that time, and gave strong support to the theory that development in the germinal sacs of the digenetic trematodes is a germinal lineage. She determined that the diploid number of chromosomes was 16 and found no reduction in the divisions of germinal cells in the germinal sacs. This observation has been confirmed in the present study.

During the summers of 1948, 1949 and 1950 at the University of Michigan Biological Station we studied the development of the mother sporocyst and rediae of *P. kellicotti* and traced the cells of the germinal line in living material. Our studies confirm most of the observations of Ameel and Chen on these stages and add new information on certain phases of their development. The study of living material gave a much clearer picture of the morphological relations of the stages in germinal development than could be obtained from sections.

MATERIAL AND METHODS

In the summer of 1948 we made some observations on natural infections of *P. kellicotti* in *Pomatiopsis lapidaria* that were obtained near Ann Arbor, Michigan, and in the summers of 1949 and 1950 we were able to carry out experimental infections in large numbers of snails of various ages from the same area, including both juveniles and adults. In none of the snails used in the infection experiments did we find any evidence of the presence of natural infections of *P. kellicotti*.

Eggs for experimental use were obtained from the lungs of laboratory infected cats and were incubated at room temperature in large stender dishes. Active miracidia were observed within the eggs in about three weeks, and hatching generally occurred as early as the fourth week. The collections of *P. lapidaria* were exposed to large numbers of miracidia to assure a plentiful supply of developmental stages. The snails were maintained satisfactorily in shallow dishes in damp soil and decomposing leaves.

The sporocysts and rediae were carefully removed from the crushed snails under a low power binocular microscope with the aid of sharp dissecting needles and small caliber pipettes. Living material was mounted in normal saline and studied with oil immersion lenses. *Intra vitam* staining with neutral red was helpful in following

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the development of the germinal cells. Material for sectioning was preserved in Bouin's fixative and stained with Heidenhain's iron haematoxylin.

OBSERVATION ON GERMINAL DEVELOPMENT

The miracidium-mother sporocyst stage.—The only observation we made on the miracidium was to check the number and position of the germinal cells. These were located in a group in the posterior half of the body (Fig. 1). The cytoplasm of some of them was seen to have fibrous extensions which attached them to the wall of the body cavity. The number of germinal cells counted in the small number of miracidia examined ranged from 4 to 9.

The youngest mother sporocyst studied (Fig. 2) was from a snail examined about 24 hours after infection. The cilia had been lost, but other structures were only slightly modified. Eight germinal cells were counted in its body cavity. Specimens recovered after two days of development were slightly larger, but showed no increase in the number of germinal cells. However, in a mother sporocyst examined after four days 13 germinal cells were counted in the somewhat enlarged body cavity (Fig. 3). In the mother sporocyst shown in figure 4 which was recovered 7 days after infection there were 5 germinal cells and 6 embryos in the 2 to 4-cell stage. There was considerable variation in the rate of development of these early stages, since in snails examined 5 to 6 days after infection we found a few sporocysts with only germinal cells and all gradations up to those with rather large embryos and only a few germinal cells (Fig. 5). It can be seen that the sporocyst shown in figure 5 had advanced in the development of the germinal material considerably beyond that in figure 4. Its enlarged body cavity contained 7 larger embryos, 3 in about the 3-cell stage, and 4 germinal cells. Figure 6 represents a sporocyst at 8 days that is in about the same stage of development as figure 5, which had 7 germinal cells that had not yet developed. A comparison of figures 2 to 6 shows that increases in size in these early stages were small compared with the rapid development of the germinal material. Most sporocysts recovered at 7 to 8 days after infection had grown considerably in size, and their greatly enlarged body cavities contained a much larger amount of germinal material (Fig. 7). Later stages were notably larger, their embryos had increased in size, and there was a gradual increase in the total number of germinal elements (Figs. 8 and 9). However, the number of germinal cells present in these and all later stages was always small.

Figure 10 represents a mother sporocyst that had reached about full-size and was almost mature. Its largest radial embryo had a well developed digestive system and

PLATE I

Germinal development in mother sporocysts of *P. kellicotti*

DESCRIPTION OF FIGURES

FIG. 1. Diagrammatic drawing of miracidium showing location of germinal cells.

FIG. 2. Mother sporocyst at one day after infection, 0.045 by 0.032 mm.

FIG. 3. Mother sporocyst at 4 days, 0.073 by 0.030 mm.

FIG. 4. Mother sporocyst at 7 days, 0.085 by 0.036 mm.

FIG. 5. Mother sporocyst at 6 days, 0.075 by 0.045 mm.

FIG. 6. Mother sporocyst at 8 days, 0.078 by 0.035 mm.

FIG. 7. Mother sporocyst at 7 days, 0.13 by 0.04 mm.

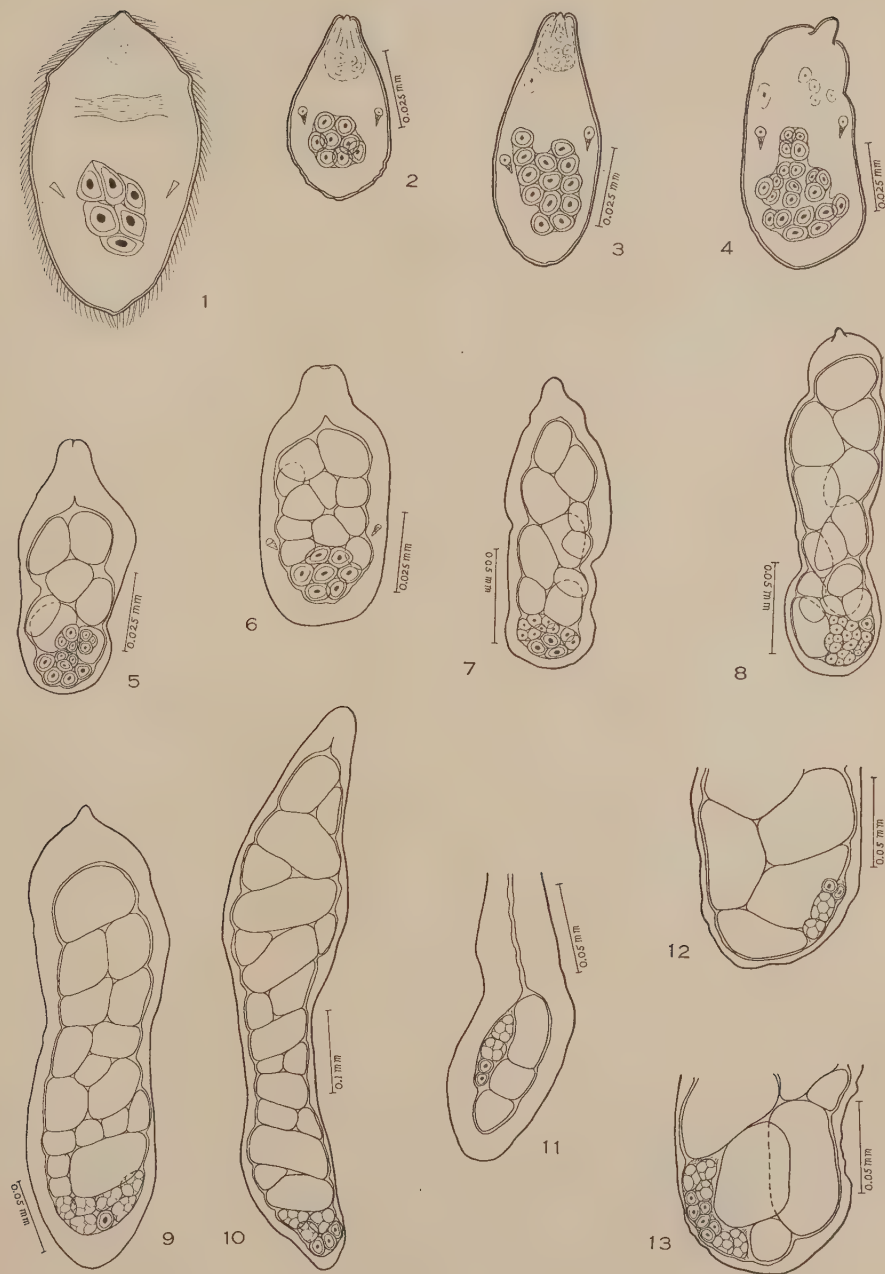
FIG. 8. Mother sporocyst at 13 days, 0.20 by 0.06 mm.

FIG. 9. Mother sporocyst at 17 days, 0.33 by 0.10 mm.

FIG. 10. Almost mature mother sporocyst at 18 days, 0.68 by 0.15 mm.

Figs. 11, 12, 13. Posterior ends of almost mature mother sporocysts showing arrangement of germinal material.

PLATE I



measured 0.14 by 0.08 mm. This sporocyst contained 19 embryos of various sizes free in its body cavity and a group of germinal elements consisting of three small embryos and three germinal cells attached at the posterior end of its body cavity. This same grouping of germinal elements, including both small embryos and germinal cells at the posterior end of the body cavity, was found in all the larger young mother sporocysts that were carefully examined (Figs. 11, 12, 13 and 14). These elements were firmly attached to each other and to the wall of the body cavity. All the larger embryos moved freely in the body cavity. The number of elements in the attached group was small in some cases (Fig. 12). In others it was somewhat larger due to the adhesion of larger numbers of embryos. For example, in one almost mature sporocyst (not figured) the attached group included two germinal cells and five embryos, the largest of which was about $30\ \mu$ in diameter. In sections of mother sporocysts of the same age as those shown in figures 11 to 14, the firm attachment of the germinal elements in these groups could be clearly seen and the germinal cells could be easily distinguished from the somatic cells of the embryos (Fig. 15). No germinal cells or attached embryos were observed in older mother sporocysts from which a number of the rediae had escaped. In 16 large mother sporocysts from 17 to 28 days old from which no rediae had yet escaped, the numbers of germinal elements, including free and attached embryos and germinal cells, varied from 20 to 30 with an average of 25.

The time after infection at which the first rediae escaped was not determined accurately. In some examinations made 28 days after infection no free rediae were found, but some of those in the sporocysts appeared about ready to escape. In two infections, 28 and 30 days after exposure to miracidia, a few free mother rediae were present. Mother sporocysts lasted for a long time in our experimental infections; some still containing embryos were found in a few snails as long as three months after infection.

First generation rediae.—It was possible in the smallest first generation redial embryos from mother sporocysts to follow and count the cells of the germinal line. Figures 16 and 17, taken from a number of drawings of these earlier stages, show respectively 2 and 4 germinal cells which by an increase in number are to form a simple germinal mass. These embryos are at about the same stage of development as those shown in section in figure 15. When the number of germinal cells had

PLATE II

Germinal development in mother sporocysts and mother rediae of *P. kellicotti*

DESCRIPTION OF FIGURES

FIG. 14. Posterior end of almost mature mother sporocyst showing arrangement of germinal material.

FIG. 15. Section through the posterior end of almost mature mother sporocyst.

FIG. 16. Very young mother redial embryo, 0.035 by 0.033 mm., showing two germinal cells.

FIG. 17. Very young mother redial embryo, 0.043 by 0.026 mm., showing four germinal cells.

FIG. 18. Mother redial embryo, at stage when primordia of pharynx and intestine are first visible, 0.048 by 0.34 mm., showing small group of germinal cells in primitive body cavity.

FIGS. 19 and 20. Mother redial embryos, 0.084 by 0.044 and 0.14 by 0.08 mm. respectively, with morula-like group of germinal cells in the primitive body cavity.

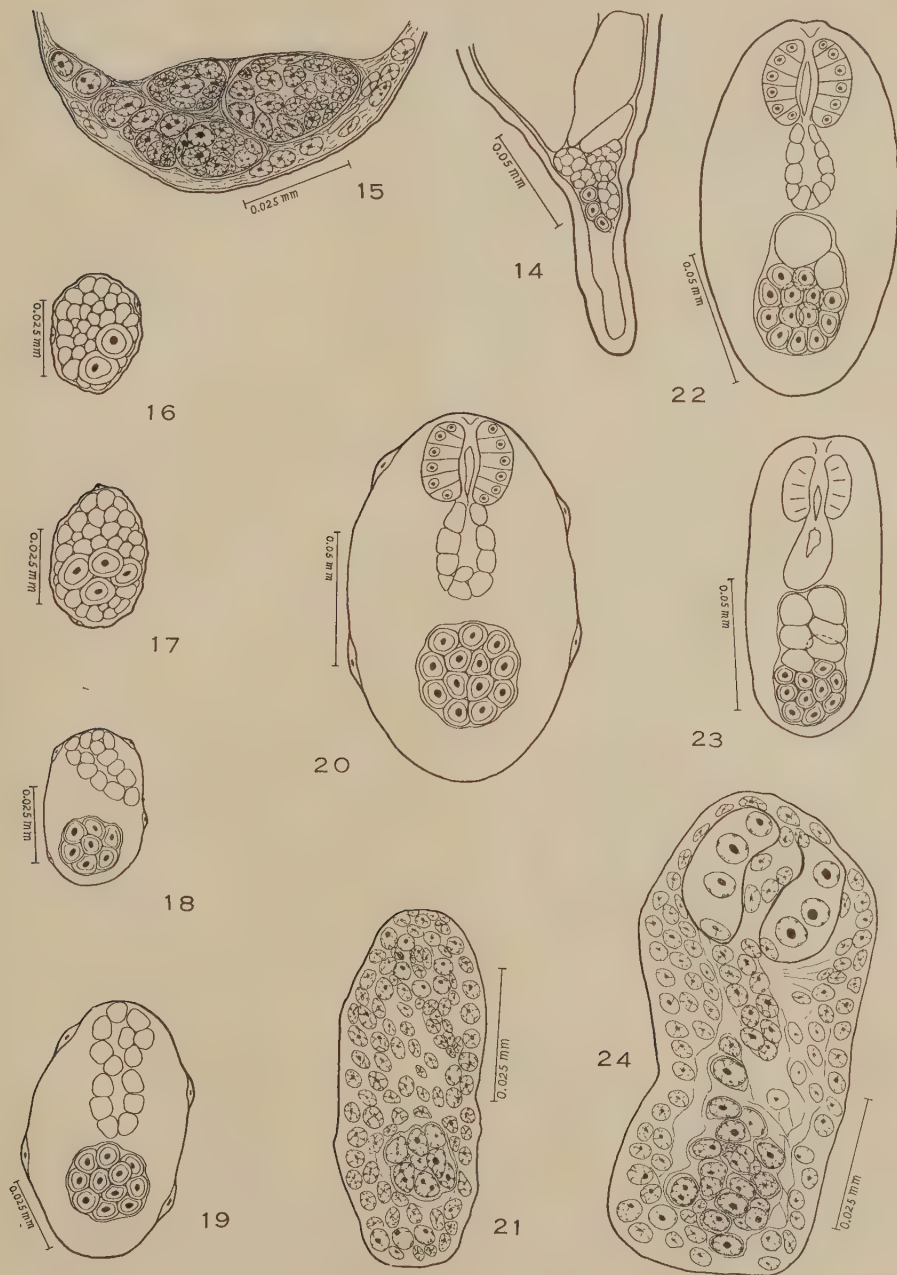
FIG. 21. Section of mother redial embryo at about the stage shown in figures 19 and 20.

FIG. 22. Mother redial embryo, 0.14 by 0.07 mm., showing earliest embryos of second generation rediae.

FIG. 23. Mother redial embryo, 0.11 by 0.05 mm., with germinal development at a slightly later stage than figure 22.

FIG. 24. Section of mother redial embryo at about the stage of Figure 23.

PLATE II



reached about 8 they were definitely enclosed in the primitive body cavity, and the primordium of the digestive system could be made out (Fig. 18). From this stage until the first embryo was formed these cells were found in slightly increased numbers and appeared as a morula-like group in the primitive body cavity back of the tip of the intestine (Figs. 19 and 20). Figure 21 shows how the group of germinal cells appears at this stage in sections. At about the time the number of germinal cells in the germinal mass reached 12 to 16 the first embryos began to develop (Fig. 22). Figures 23 and 24 show the comparison of a slightly later stage from living material and sections. Before the mother redial embryos reached their largest size in the mother sporocysts their body cavities had expanded considerably and the majority of their germinal cells had developed into embryos (Figs. 25, 26 and 27). In the smallest of the free mother rediae that were found, development of the germinal material was at about the same stage as in the larger rediae in the mother sporocysts (cf. Figs. 27 and 28). These stages showed only a slight increase in the total number of germinal elements as compared with the earlier stages when only a morula-like mass of germinal cells was present. Larger free mother rediae tended to be contracted with truncated posterior ends and had larger pharynges and expanded intestines (Fig. 29). These rediae contained only a few germinal cells, but there was an increase in the number of embryos in the enlarged body cavity (Fig. 29). Figure 30 shows an almost mature mother redia; the largest daughter redial embryos in it were well developed and appeared almost ready to escape; three germinal cells and 30 embryos were counted. Though we were unable to determine exactly at what stage germinal cells disappeared, neither they nor small embryos were observed in older mother rediae from which some of the daughters had escaped.

Counts of the germinal elements in large immature and mature mother rediae varied considerably. Thirty-seven, 38 and 38 daughter redial embryos were counted in three well-developed mother rediae in which no germinal cells were found. The counts were less in some cases where the rediae were almost mature.

Second generation rediae.—The development of the germinal cells was traced in very small embryos of second generation rediae which were removed from the mothers. We made a number of measurements both of free germinal cells in the mother rediae and of those in the smallest daughter redial embryos; they varied in size from 10 to 13 μ . Development of the germinal cells in the earliest embryos of the second

PLATE III

Germinal development in mother and daughter rediae of *P. kellicotti*

DESCRIPTION OF FIGURES

FIGS. 25, 26, and 27. Mother redial embryos, 0.14 by 0.06 mm., 0.15 by 0.06 mm., and 0.16 by 0.05 mm., respectively, showing increase in the number of daughter redial embryos and decrease in the number of germinal cells.

FIGS. 28 and 29. Early stages of free mother rediae, 0.12 by 0.08 mm. and 0.18 by 0.09 mm. respectively.

FIG. 30. Free mother redia, 0.38 by 0.29 mm., almost fully developed with daughter rediae almost ready to escape.

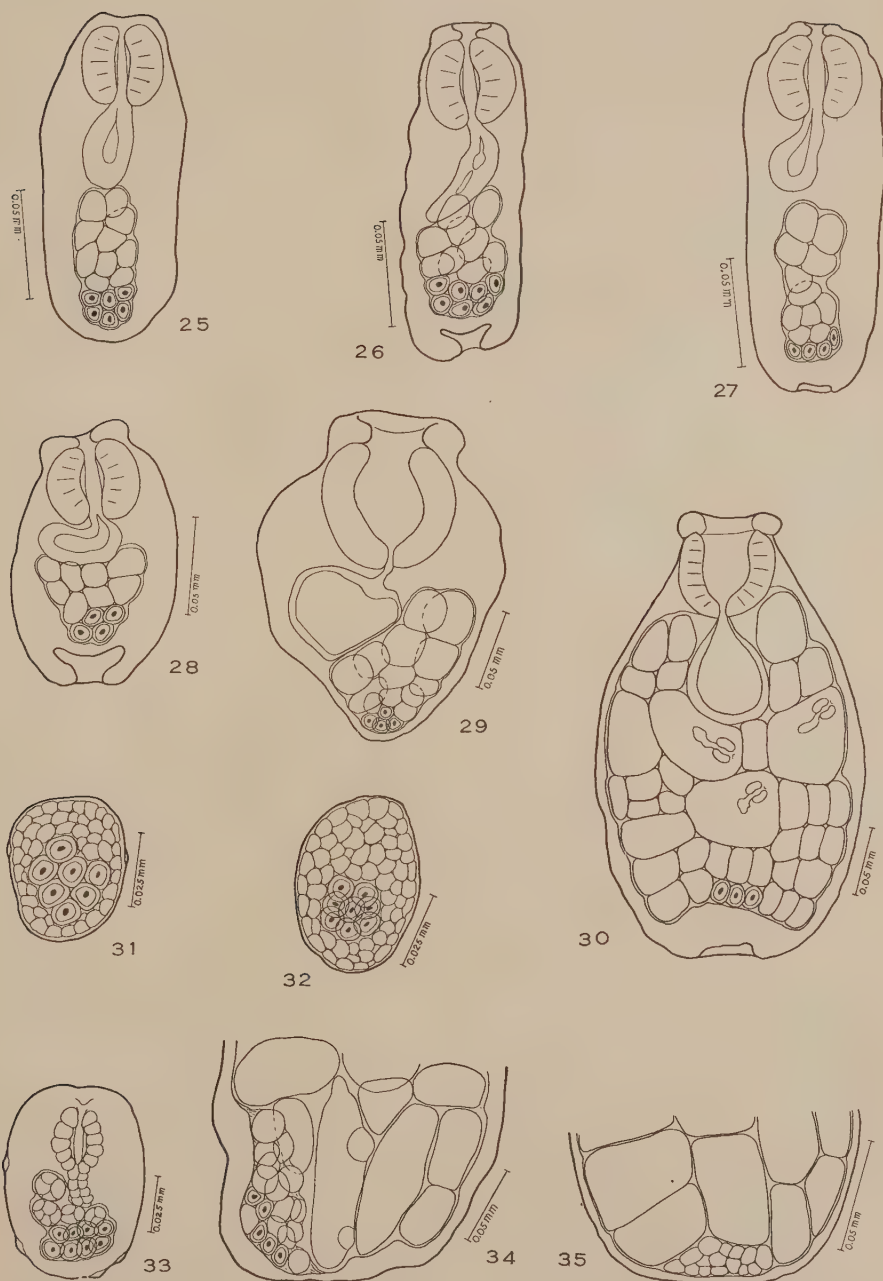
FIGS. 31 and 32. Very immature daughter redial embryos, 0.036 by 0.026 mm. and 0.040 by 0.030 mm. respectively, showing groups of germinal cells in primitive body cavity.

FIG. 33. Daughter redial embryo, 0.082 by 0.053, with first cercarial embryos.

FIG. 34. Posterior end of almost mature daughter redia, 0.60 by 0.27 mm., showing well developed complex germinal mass.

FIG. 35. Posterior end of old daughter redia, 0.50 by 0.27 mm., showing germinal mass still persisting.

PLATE III



generation rediae appeared to be exactly like that of the first generation. Figures 31 and 32 show stages before the primordia of the pharynx and intestine could be made out. In the stages between figure 32 and the formation of the first cercarial embryo, the germinal cells were in a morula-like mass immediately back of the tip of the intestine just as at the corresponding stage of the first generation rediae. In fact, germinal development in the two redial generations appears to be identical up to the time they are ready to escape from their parent germinal sacs. Figure 33 shows a daughter redial embryo in which a few of the germinal cells had developed into cercarial embryos.

We were particularly anxious to study germinal material in free daughter rediae. Since they produce such large numbers of cercariae over a long period of time, we felt that the mechanism of germinal development in second generation rediae must differ fundamentally from that of the first generation in which the multiplication of germinal cells is limited and comparatively few individuals are produced. Unfortunately the number of experimentally infected snails that survived up to this stage was so small that we were able to make adequate studies of larger free daughter rediae only. These, as first shown by Ameel (1934), can be distinguished from mother rediae by the relatively small size of the pharynx and also usually tend to be more elongate than the mother rediae. Later, of course, they contain cercarial embryos but even before the structure of these can be determined with certainty in the larger daughter rediae, the number of embryos is considerably greater than in the mother rediae.

Studies of larger daughter rediae showed that in contrast to the mother rediae a large complex germinal mass containing both unicellular and multicellular components was present attached at the posterior end of the body cavity. Figure 34 shows this mass in a daughter redia measuring 0.60 by 0.27 mm. and containing about 70 cercarial embryos the largest of which showed the differentiation of suckers and the beginning of a tail. Another daughter redia measuring 0.85 by 0.32 mm. with a germinal mass similar to that shown in figure 34 contained almost 100 cercarial embryos none of which showed the differentiation of suckers and a tail. In a somewhat contracted younger daughter redia measuring 0.40 by 0.20 mm., the large germinal mass contained about as many multicellular components as the one shown in figure 34 but only four germinal cells could be made out.

Mature and old daughter rediae from natural infections.—In the 1282 specimens of *P. lapidaria* examined during the summer of 1948 there were seven mature and old infections of *P. kellicotti*. These evidently had been acquired the previous summer and had lasted over the winter. Only daughter rediae were recovered and almost all of them were either mature or old. In six of these infections 11, 19, 21, 25, 40 and 59 daughter rediae were counted. A number of these were studied and in them were found germinal masses containing both unicellular and multicellular components attached at the posterior end of the body cavity. In the oldest rediae these masses were rather small and somewhat flattened and in no case were they as large as those in the younger daughter rediae from the experimental infections. However, some of them at least were still producing embryos as indicated by the presence of free embryos little if any farther advanced than the larger multicellular components of the germinal masses. Figure 35 shows the germinal mass of an old pigmented daughter redia, 0.50 by 0.27 mm.

DISCUSSION

The general course of the life cycle of *P. kellicotti* was outlined by Ameel (1934). There is little to add from the present studies. Minor variations in the time at which the different stages first appeared can be ascribed chiefly to differences in temperature. Our counts of the numbers of rediae produced in the mother sporocysts and first generation rediae are somewhat larger than those given by Ameel who used very small snails in his experimental infections. We found also, contrary to his experience, that mature as well as juvenile snails could be infected experimentally. A comparison of our observations with those of Chen (1937) shows no significant differences.

The finding of an average number of 25 germinal elements in mother sporocysts that were almost mature indicated that their potential reproductive capacity exceeds that figure. Counts indicated that the number of daughter rediae that could be produced by mothers was somewhat larger. Using conservative estimates of 25 and 30 respectively, a reproductive potentiality is indicated that might produce as many as 750 daughter rediae from the infection of a snail with one miracidium. It is obviously impossible that such a large number of rediae could develop and produce cercariae in the digestive gland of such a small snail as *P. lapidaria*. The few actual counts that have been made of the number of mature daughter rediae in natural infections of *P. kellicotti* are surprisingly low. Our counts of six infections ranged from 11 to 59 with an average of 29. Ameel in 15 natural infections in which counts were made gave a range of from 9 to 62 with an average of 22. This is a very striking illustration of what appears to be a general correlation in digenetic trematodes; namely, that the number of secondary germinal sacs that actually develop is determined by the space and food supply in the intermediate host rather than by the reproductive potential.

No one appears to have actually counted the output of cercariae from snails infected with *P. kellicotti*. The daughter rediae are larger than the mother rediae and contain more embryos. Ameel (1934) stated that some second generation rediae contained as many as 20 to 30 fully developed cercariae. Our few counts of the cercarial embryos in rediae are also large. Furthermore, infections that were obviously over a year old still produced cercariae in large numbers. It is evident, therefore, that the second generation rediae of *P. kellicotti* have developed the ability to produce large numbers of cercariae to meet the hazards of transfer by a second intermediate host.

From our studies, the course of germinal development in *P. kellicotti* appears about as follows. Division of the cells of the germinal line has already started in the miracidium to produce a group of about 8 in its body cavity. Early in the development of the mother sporocyst, there is a slight increase in the number of these cells before any of them start to develop into embryos. Soon all except a few of the germinal cells have produced embryos. These remaining cells and a few of the smallest embryos are found attached at the posterior end of the body cavity of the mother sporocyst. Division of these cells and the formation of new embryos continue throughout the development of the mother sporocyst. This center of multiplication of germinal cells persists at least until the first mother rediae escape, and might be considered as a very simple, primitive type of germinal mass. In the mother redial embryos at about the time that the cells of the digestive system become

well defined, the cells of the germinal line have formed a morula-like group in the primitive body cavity. Soon the most anterior cells in this germinal mass develop into embryos, and most of them have become embryos by the time the young mother rediae have escaped from the mother sporocyst. However, a few germinal cells attached at the posterior end of the body cavity continue to multiply and produce embryos. All are used up shortly after the mother rediae become fully developed. Therefore, the mechanism of germinal production in the mother rediae resembles that of the mother sporocysts and in both, with a rather brief period of multiplication of germinal cells, only a comparatively small number of embryos is produced. In early stages of the daughter rediae development of the germinal material is like that of the mothers. However, in later stages, instead of the germinal cells having only a limited period of multiplication, they are found in a large persistent germinal mass which is still present and producing new embryos in mature and old daughter rediae. This very great extension of the period of multiplication of germinal cells provides for the production of large numbers of cercariae. This is the first case in which it has been clearly demonstrated that the mechanism of germinal development differs in succeeding generations of rediae of the same species.

Further comment on germinal development in the mother sporocyst is worthwhile. As noted above, the center of multiplication of germinal cells at the posterior tip of the body cavity of the mother sporocyst of *P. kellicotti* might be considered as a primitive simple germinal mass. It is very significant that in *Halipegus* in which the mother sporocyst produced large numbers of rediae, a large persistent germinal mass, very similar to that of the rediae, develops at the posterior end of the body cavity in the mother sporocyst (Ameel, Cort and Van der Woude, 1949). These similarities in the mechanism of germinal development in mother sporocysts and rediae offer strong evidence for their fundamental homology.

SUMMARY

Studies have been made of the germinal development in the mother sporocyst and two generations of rediae of *Paragonimus kellicotti* Ward, 1908. Material was obtained largely from experimentally infected *Pomatiopsis lapidaria* though the initial study was made on material collected from naturally infected snails. In the miracidia and very young mother sporocysts the germinal elements consist of a cluster of a small number of germinal cells. These give rise to a limited number of embryos and a very simple primitive type of germinal mass which persists until after the escape of the first mother rediae. The mechanism of germinal production in the mother rediae is similar to that in the mother sporocyst and only a small number of daughter redial embryos is produced. The development of germinal material in the early stages of the daughter redial embryos resembles that in the mother rediae. However, in later stages there is a large germinal mass which consists of unicellular and multicellular components and persists even in old daughter rediae thus accounting for the production of large number of cercariae.

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MEGACIRRUS MEGAPODII N. G., N. SP., A CESTODE FROM THE
MALAYAN BRUSH TURKEY, MEGAPODIUS LAPEROUSE SENEX
(CESTODA: DILEPIDIDAE)

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Among some parasites collected by Mr. Rollin H. Baker in the South Pacific during World War II and sent to this laboratory for identification was a vial containing a group of tapeworms from *Megapodius laperouse senex* taken on Palau Island, Peleliu atoll, in the Malay Archipelago. The cestodes were not in very good condition, and only one of several similar specimens, when stained with carmalum, was satisfactory for descriptive purposes. This one specimen appears to represent a new genus and species in the subfamily *Dilepidinae* Fuhrman, 1907.

Grateful acknowledgement is accorded to Dr. Asa C. Chandler for helpful suggestions and criticisms.

Megacirrus n. g.

Diagnosis: Dilepidinae. Scolex with rostellum armed with double row of similar hooks. Suckers unarmed. Genital organs single, ducts unilateral, passing between longitudinal excretory vessels. Cirrus pouch without seminal vesicle, very long, extending to or beyond excretory canals on aporal side in some mature proglottids. Testes numerous, in posterior region of proglottid. Ventral longitudinal excretory canals broad, dorsal vessels narrow and sinuous. Uterus saciform, deeply lobed, not breaking up into egg capsules. Parasites of birds. Type species, *M. megapodii*.

Megacirrus megapodii n. sp.

Diagnosis: Strobilae up to 30 mm. long; musculature well developed. Proglottids up to about 465 in number. Scolex 370 μ long and 320 μ wide at region of suckers. Suckers 180 μ in diameter. Rostellar sac 200 μ in diameter, tapering to cone below neck region. Rostellum armed with 2 rows of hooks, approximately 22 in each row, equal in size and shape, with second row recovered at tip (Fig. 5). Second row set 15 μ posterior to anterior border of first row. Hooks 100 μ long, with long blade (64 μ), root 32 μ long and short guard. Neck not discernible, strobila approximately 180 μ in diameter just behind scolex. Proglottids broader than long, from 120 μ long \times 780 μ wide in region of mature proglottids up to 180 μ \times 830 μ in region of gravid proglottids.

Testes 14-15 in number, varying from round (40 μ in diameter) to oval (64 μ \times 35 μ), concentrated mainly along posterior border of proglottid, both dorsal and ventral to cirrus pouch, extending laterally beyond lateral borders of excretory vessels on dorsal side. Cirrus pouch approximately 35 μ in diameter, with maximum lengths up to 495 μ , extending from genital atrium medially in slight "S" curve, slightly tortuous along anterior border of proglottid, sometimes reaching outer border of aporal ventral excretory duct, either dorsal or ventral to it. Cirrus up to 12 μ in diameter in region of genital atrium, spineless, without seminal vesicle, protruded portion up to 340 μ long. Vas deferens a coiled mass dorsal to aporal end of ovary. Vagina 9 μ in diameter, tortuous, parallel and dorsal to cirrus pouch. Genital ducts unilateral, passing between longitudinal excretory canals on right side. Genital atrium small, with thick, fleshy walls forming a genital papilla about 76 μ in diameter on lateral border of proglottid. Seminal receptacle about 54 μ in diameter, approximately median, dorsal to cirrus pouch, usually dorsal to vitelline gland but sometimes ventral. Ovary lobed, narrowing to isthmus in region of seminal receptacle, extending laterally between dorsal and ventral vessels, with a lobe extending ventral to ventral excretory vessel on aporal side; maximum antero-posterior width approximately 80 μ . Vitelline gland compact, approximately 135 μ \times 40 μ , situated in posterior median part of proglottid, its borders overlapped by testes both dorsally and ventrally. Ventral longitudinal excretory canals about 45 μ in diameter, dividing body into almost equal thirds in segments with mature

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testes, but in older segments lateral areas a little narrower than median area. Transverse ducts inconspicuous. Dorsal excretory canals 4 to 5 μ in diameter, tortuous, lying dorsal to ventral excretory ducts, their sinuities extending both lateral and median to them. Uterus sacciform, deeply lobed, not breaking up into egg capsules, approximately $495 \mu \times 180 \mu$, extending laterally between and beyond longitudinal excretory canals.

In the maturing proglottids the male genitalia precede the female in development, but the developing ovary and vitelline gland were not discernible until a fair degree of maturity of these organs had been reached, due probably to imperfect fixation. The eggs could not be studied satisfactorily in the specimen available, since no fully ripe proglottids were present.

This worm is unique among the Dilepididae in the length of the cirrus pouch, which in some proglottids extends beyond the aporal excretory ducts.

EXPLANATION OF PLATE

FIG. 1. Scolex.

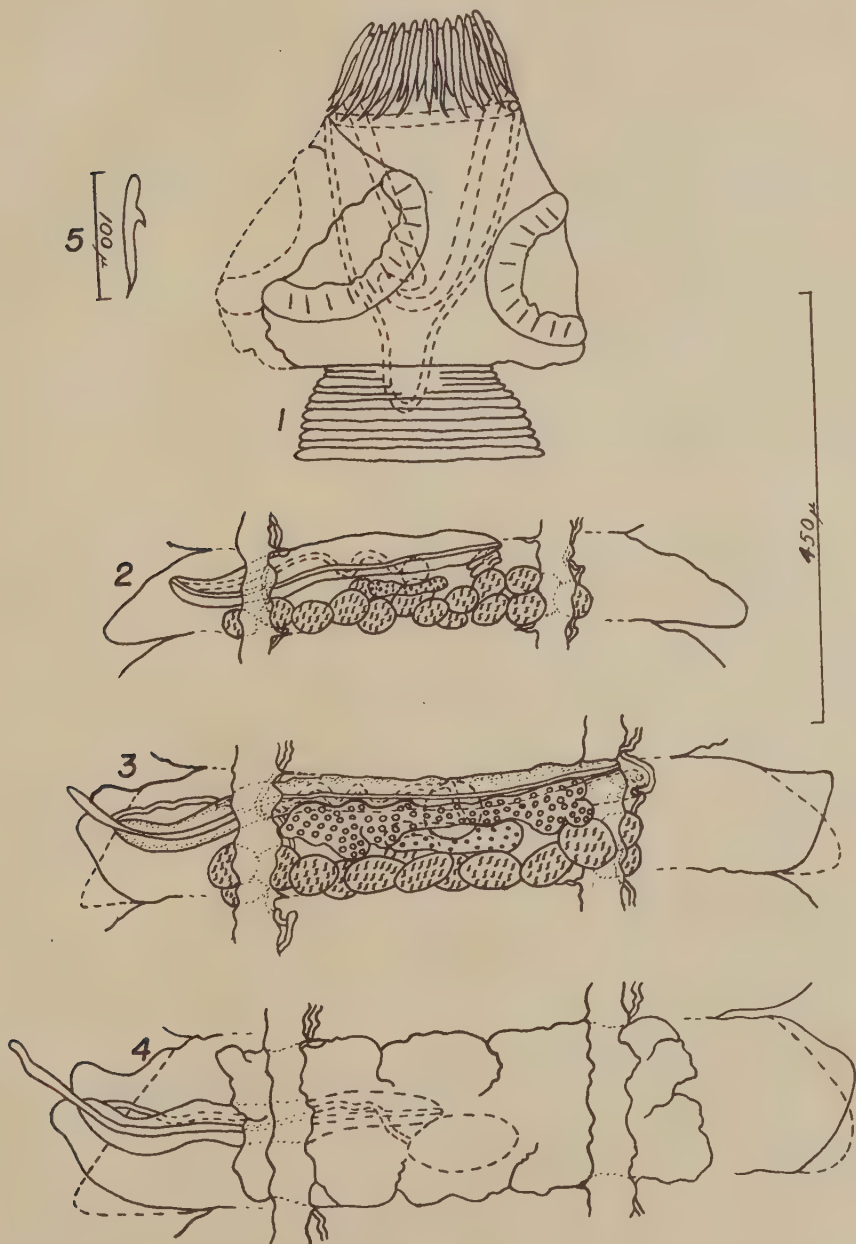
FIG. 2. Young proglottid, ventral view, with female system just beginning to develop, but male system functional.

FIG. 3. Fully mature proglottid, ventral view.

FIG. 4. Gravid proglottid, ventral view, showing lobulated, sacciform uterus.

FIG. 5. Rostellar hook.

PLATE I



STUDIES ON EXPERIMENTAL CHAGAS' DISEASE IN MICE IN RELATION TO CHEMOTHERAPEUTIC TESTING

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The empirical approach to the discovery of new compounds active against *Trypanosoma cruzi* necessitates extensive *in vivo* screening. Experimental Chagas' disease in mice is commonly used in such work, but little detailed information on the use of this infection in chemotherapeutic testing has been published. In all of the earlier work, reviewed by Stein (1933), as well as in more recent investigations (Jarpa *et al.* 1949, 1950 a, b; Goodwin *et al.* 1950) the blood of infected animals has been used as inoculum. The growth of *T. cruzi* in culture, however, has considerably simplified the preparation of quantities of homogeneous inoculum required for the infection of large numbers of animals. It is the purpose of this article to report certain observations on the course of experimental Chagas' disease in mice, induced with cultural inocula, and to describe methods for the chemotherapeutic testing of new compounds for activity against this infection.

MATERIALS

Two strains of *Trypanosoma cruzi* Chagas, 1909 were used during these investigations:

Strain "A" was obtained in April 1947, from Dr. Robert J. Schnitzer of the Chemotherapeutic Laboratory of Hoffman-LaRoche, Inc., Nutley, N. J. The original source of this strain is unknown. It came to Hoffman-LaRoche in August, 1942, from the Army Medical School through the American Type Culture Collection, and its virulence was enhanced by rapid passage through 3-week-old Webster mice (Lewis, 1946). In September, 1947, it was put into culture.

Strain "B" ("Brazil"), was obtained in November, 1946 from Dr. Theodore S. Hauschka of the Institute for Cancer Research of Lankenau Hospital, Philadelphia. This strain, isolated from a Brazilian patient in 1942 and maintained in culture at the National Institutes of Health, was obtained by Dr. Hauschka in December, 1945.

Both of these strains were first kept in the medium devised by Johnson (1947), comprising an agar base containing beef infusion, Neopeptone (Difco) and 10% defibrinated rabbit blood, overlaid with Locke's solution. This medium was later modified by use of veal instead of beef infusion, and still later by substituting Bacto brain heart infusion agar as the base, to which the blood and Locke's solution were added as above. No differences in abundance or rate of growth of *T. cruzi* were observed when these modifications were made.

The mice used were, with the exception of one test, 3-week-old Webster Swiss purchased from Mr. Dow Hover, Germantown, N. Y. The excepted test was run on adults and also included A-mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

METHODS

In all tests but two (in which adults were used) the mice were 3 weeks old when infected. Usually 20 animals of the same sex were used in each group. In all tests

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but one, in which the inoculum was infected blood, the mice were infected by intraperitoneal injection of overlay from 2-week-old cultures of *T. cruzi*; the infective dose was approximately 30 million organisms in most of the tests on which evaluations of therapeutic activity were based. Smaller numbers (20, 15 and 10 million) were used on some of the earlier tests but could not be relied upon to produce high mortality. When 3-week-old mice were infected with 30 million cultural forms in 0.3 ml. of overlay, deaths sometimes took place as early as the 10th day.

It was considered desirable, in screening, to complete the medication of the test animals before the onset of mortality in the controls. If the first dose of drug was given four days after injection, 6 consecutive daily doses could be administered before deaths began to occur among the untreated mice. The daily dose of drug usually consisted of a single *intraperitoneal* injection of the equivalent of the acute *intravenous* LD₅₀ of the compounds to be tested. In most cases this amount was well tolerated although with some compounds the dosage had to be reduced. On evaluation tests of active compounds, doses greater and smaller than the intravenous LD₅₀ were used and the time of medication and length of the medication period were varied; some drugs were also administered orally by intubation.

OBSERVATIONS

When strain "A" of *T. cruzi* was first tested, using infected blood as inoculum, a mortality of 90 per cent was obtained with the first deaths occurring in 15 days (Table 1). Five months later the strain was put into culture and in the first two tests, employing culture as inoculum, 100 per cent mortality was obtained, all deaths occurring between 10 and 34 days.

TABLE 1.—Course of infection of *T. cruzi* ("A" strain) in Webster mice

Date of test	Age of mice	Period of mortality (days)	Median survival time (days)	Per cent surviving at 60 days	Mean survival time (days)
In males					
5-29-47*	3 weeks	15—	29	10	31
10-23-47	adult	10-34	13	0	15
11-20-47	3 weeks	10-25	13	0	15
In females					
12-11-47	3 weeks	17—	22	15	28
3-11-48	"	12—	15	10	15
3-18-48	"	12-35	14	0	17
6-3-48	"	14—	18	20	26
8-3-49	"	20—	..	80	42

* The inoculum for this test was blood from infected mice; on all other tests the inoculum was 30 million cultural forms per mouse, in overlay from Johnson's medium.

Strain "A" maintained high virulence in mice for the first nine months in culture. During this period the infection was also uniformly fatal to puppies. When tested in puppies one year after being put into culture, however, strain "A" was found to be non-infective and several subsequent attempts to infect puppies met with failure. When subsequently tested in mice it was found that there had been a parallel diminution in virulence, so that only 20 per cent mortality occurred and the onset was a week later than expected.

No explanation of this change in behavior was immediately apparent, although it was noted that there was much less tendency to form rosettes in the avirulent "A" strain cultures than in the "B" strain cultures which have undergone no change

in pathogenicity over a period of years. The two cultures were maintained side by side in the laboratory, the media and other conditions being identical.

Starting with mice infected with the avirulent culture of strain "A" serial syringe passage was again established. At first the animals did not die and did not reach a parasitemic peak suitable for transfer until 3 to 4 weeks after infection. After several passages, however, parasitemia occurred earlier and at higher levels and some of the mice died. By the end of a year deaths were consistently occurring in about 3 weeks and at the end of the second year, during which transfers were made at approximately weekly intervals, the mean survival time was 12 days.

Although the periods of mortality and survival percentages with strain "A" did not indicate that adults differed from weanlings in susceptibility to infection (Table 1), a test with strain "B," run simultaneously on adults and weanlings, did show

TABLE 2.—*Course of infection of T. cruzi ("Brazil") in Webster mice*

Sex of mice	Size of inoculum	Period of mortality (days)	Median survival time (days)	Per cent surviving at 60 days	Mean survival time (days)
Males	10 million	28—	..	90	57
Males	15 million cultural forms per mouse	21—	36	30	40
		25—	35	20	37
		21—	28	20	38
		19—	22	15	29
		22—	28	15	34
		19—	29	35	39
		24—	38	25	42
		Averages	31	23	37
Males	30 million cultural forms per mouse	10-31	15	0	17
		11-34	17	0	17
		10-30	20	0	21
		13-24	16	0	17
		13-66	17	15	22
		11-17	15	0	15
		13-34	17	0	19
		Averages	17	2	18
Females	30 million cultural forms per mouse	21—	27	5	33
		17-33	19	0	20
		18—	36	10	39
		10-23	11	0	11
		13-19	15	0	15
		15-36	22	0	22
		16—	24	10	29
		Averages	21	3	24

appreciable differences, the median survival times being 24 and 18 days, respectively. At 30 days all of the young mice had succumbed but 30 per cent of the adults were surviving.

In experiments with "B" strain it was found that the size of the inoculum influenced both the time and percentage of mortality considerably (Table 2). It was found that an inoculum of more than 15 million organisms is necessary to produce mortality of at least 85 per cent. It was also noted that there was rather good correlation between the time of onset of mortality, the median and mean survival times and percentage survival to 60 days. If deaths occur before the end of the second week after infection, the loss of half of the animals by the end of the third week and an eventual 100 per cent mortality is predictable.

It was also apparent that male mice are generally more susceptible than females, the onset of mortality being earlier and the mean survival time less. Because male and female mice were not used on the same tests, direct comparisons with identical

inocula and other conditions could not be made but certain deviations from expected behavior occurred. Occasionally, mortality may take place as early in females as in males (10 days), or the deaths may occur more rapidly in females (13 to 19 days) than they do in males (13 to 34). Male mice showed a tendency to fight among themselves as they matured. Although their belligerency was not a problem during the 60 day period of the routine tests reported here, this behavior was found to compromise survival data in long term experiments in which the observation of cured

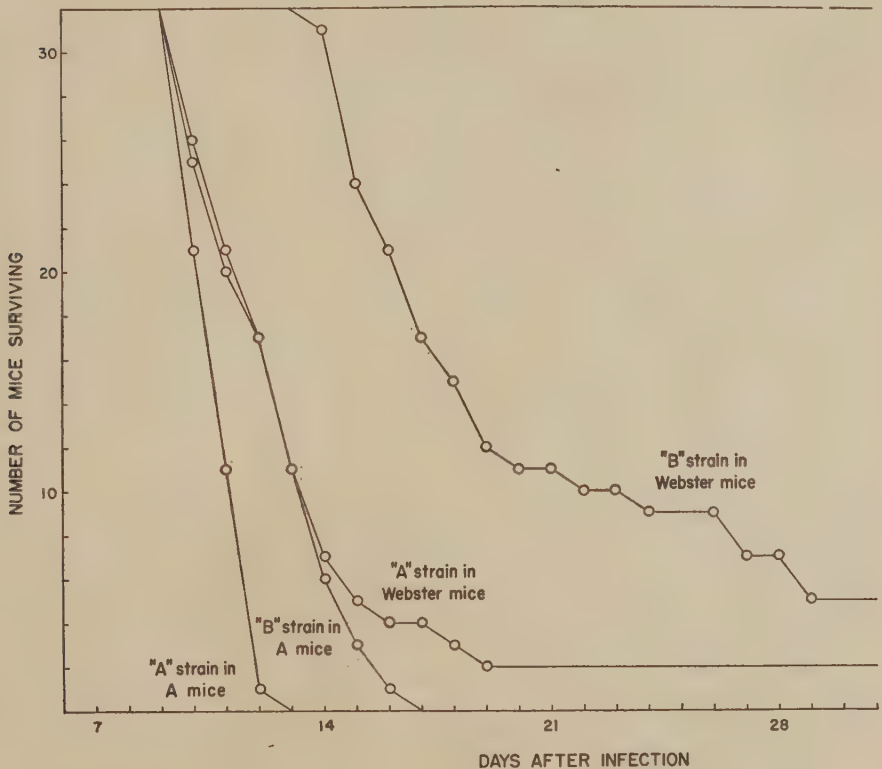


FIG. 1. Survival of adult Webster and A-mice with infections of "A" and "B" strains of *T. cruzi*.

animals for a 6 month period was desired. Eventually, therefore, the use of males was discontinued; female survivors at the end of the 60 day tests could be kept for further observation without this complication.

Prior to the diminution in virulence of "A" strain, one experiment was designed to compare the "A" and "B" strains of *T. cruzi* in adult Webster and A-mice. The inoculum was 30 million culture forms per mouse in each case. The results indicated that "A" strain *T. cruzi* was more pathogenic than "B" strain in both A-mice and Webster mice and that adult A-mice were more susceptible than adult Webster mice to both "A" and "B" strains of *T. cruzi* (Figure 1). The difference in susceptibility of the two strains of mice did not appear to be sufficient to warrant special

procurement of the slightly more susceptible A-mice. Strains "A" and "B" of *T. cruzi* did not differ in their sensitivity to chemotherapeutic agents of either the bisquinaldine (Bayer 7602) or 8-aminoquinoline (Pamaquin) types of compounds.

DISCUSSION

The greater susceptibility of juveniles to Chagas' disease has long been recognized in man and has been studied in a number of experimental animals. Culbertson and Kessler (1942), working with a less pathogenic strain of *T. cruzi* than ours, found that animals below 25 days old invariably succumbed to the infections while those above that age usually survived; such differential mortality would not be so readily observed with strains which cause high mortality even among adults. It is probable that differences in susceptibility between various ages would be more accentuated during the period prior to weaning than during the period thereafter. Investigators who have used rats have usually found that heavy infection could be established consistently only in very young animals, i.e. below the age of weaning (Kolodny, 1939; von Brand *et al.*, 1949).

The degree of difference in susceptibility between adults and young with any given infection is apparently dependent on the behavior of the strain of parasite in the species and strain of host used. Strains of *T. cruzi* which kill adult mice, e.g. "Wellcome" and "Brazil" (Hauschka, 1949), may have to be used in very young rats to produce infections and in 8 day old rats they may ("Brazil") or may not ("Wellcome") cause fatalities (von Brand *et al.* 1949). On the other hand the strain used by Culbertson and Kessler (1942) produced infections in mice of "distinctly lower intensity than those in rats."

Mazzotti (1940) failed to establish correlation between the size of inocula varying from 800 to 8,000 organisms originating from triatome feces and the severity of *T. cruzi* infections in mice, but our observations suggest that, at least with the strains we studied, a certain minimum number of culture forms must be injected in order to produce high mortality consistently. It is apparent that more than 15 million organisms per mouse are necessary to produce a predictable mortality of at least 85 per cent. Hauschka, Saxe and Blair (1947) used doses of 16-25 million and Hauschka (1947) used 30-40 million *T. cruzi* to produce experimental infection.

Where there is less than 100 per cent mortality the mean survival values are arbitrary figures dependent on the time of termination of the experiment, which was 60 days for most of our screening tests. Data from tests in which the control survival was greater than 15 per cent were not used in estimating drug activity.

The possibility of differences in susceptibility between the sexes of experimental hosts has been suggested by reports on certain related protozoa, e.g. *T. equiperdum* (Poindexter, 1933), *T. lewisi* (Perla and Marmorston-Gottesman, 1930) and *L. donovani* (Culbertson, 1941). With this in mind, it was considered desirable that the animals used for chemotherapeutic evaluation be homogeneous as to sex as well as to age and stock. Hauschka (1947) made a quantitative study of sex of host as a factor in experimental Chagas' disease and presented ample evidence that males were consistently more susceptible than females.

The use of the intravenous LD₅₀ intraperitoneally as the arbitrary initial dose of drug for screening was adapted from Goodwin and Marshall (1945) who used doses "equivalent to one intravenous LD₅₀" subcutaneously in testing monoamidines

for chemotherapeutic activity. We felt, however, that the more rapid absorption and distribution of drug following intraperitoneal injection was desirable in obtaining the maximum qualitative therapeutic effect. Experience with many quinoline and quinaldine derivatives indicates that the intraperitoneal administration of the intravenous LD₅₀, either as a single dose or as a series of daily doses, does not induce evident toxic effects. (Goble, 1949, 1950.)

In choosing a medication period which commenced 4 days after infection, rather than beginning immediately after inoculation, a compromise was made between screening for compounds of low activity, which might be effective only during the incubation period, and a search for compounds of efficacy in well established infections. Testing against the later stages of the disease was reserved for those compounds which were exceptionally effective when administered according to the routine regimen. Few drugs showed activity on delayed administration. At the beginning of the program none was known, so that screening for this type could not be undertaken with any assurance of success. Earlier medication made possible the detection of moderate and weak activity which furnished leads to new groups of trypanocidal compounds.

The methods described above differ somewhat from those employed by contemporary workers who have used blood of infected mice as inoculum and have judged drug activity in terms of parasitemia. The daily examination of blood during extensive tests is a tedious procedure necessitated by the occurrence, with some *T. cruzi* strains, of spontaneous recoveries and transitions to chronic infection. With mortality below 50 per cent the calculation of mean survival time is arbitrary and an estimate of median survival time impossible. With a strain of *T. cruzi* which produces consistently high mortality, however, the use of these criteria, as well as percentage survival to 60 days, in the evaluation of drug activity, is conservative of time; it is much easier to count mice than trypanosomes.

Although singular success has been achieved in the perpetuation of consistent virulence in "Brazil" strain cultures, it is possible that the behavior of other strains may differ considerably depending on genetic constitution and previous adaptation to other media or animals. The satisfactory results obtained during four years of chemotherapeutic testing are probably attributable, in large part, to the fortunate selection of an adaptable strain and a favorable cultural method.

ACKNOWLEDGMENTS

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STUDIES ON THE HELMINTH FAUNA OF ALASKA. VII—ON SOME
HELMINTHS FROM ARCTIC MARMOTS WITH THE
DESCRIPTION OF *CATENOTAENIA REGGIAE*
N. SP. (CESTODA: ANOPLOCEPHALIDAE)

ROBERT RAUSCH¹

Among Alaskan mammals examined for helminthic parasites during 1950 was a series of marmots, *Marmota caligata broweri* Hall and Gilmore, 1934, from the Brooks Range, Arctic Alaska. The animals were collected a few miles south of Tolugak Lake (lat. 68° 24' N., long. 151° 26' W.), and from the vicinity of Kanayut Lake, about 15 miles northeast of Tolugak Lake. This distinctive marmot has been hitherto represented in collections only by the three specimens comprising the type material. In addition to greatly extending the known range of this form, the collection of these specimens also contributes the first information on Brooks Range marmots (Rausch, 1950).

Three species of helminths occurred frequently in the twenty-odd animals examined. They were *Ascaris laevis* Leidy, 1856, *Diandrya composita* Darrah, 1930, and an undescribed species of *Catenotaenia* von Janicki, 1904. Tiner (1951) has recently redescribed *A. laevis*, partially on the basis of Alaskan material. The writer has recovered this ascarid also from ground squirrels, *Citellus parryi barrowensis* (Merriam), from Umiat, which is located on the Colville River at the north edge of the Arctic Plateau, and from Tolugak Lake. It is uncommon in this host, however, and is therefore considered essentially a parasite of marmots. Cestodes tentatively determined as *D. composita* have been reported from *Marmota c. caligata* (Eschscholtz) in Alaska by Philip (1938). Philip also reported *Ascaris tarbagan* Shul'ts, 1931 from *Marmota monax ochracea* Swarth, collected in central Alaska. A more complete coverage of the parasitic helminths of Alaskan sciurids will be published when adequate data have been collected.

According to a review of the literature, and the Host Catalogue of the Zoological Division, Bureau of Animal Industry, no species of *Catenotaenia* has been recorded from marmots, although two species have been recorded from other sciurid hosts. The Brooks Range material is readily differentiated from previously-described species, and is described herewith as new.

Catenotaenia reggiae n. sp.

(Figs. 1-4)

Diagnosis: Anoplocephalidae. Maximum strobila length 360 mm.; greatest width, attained in gravid segments, 3 mm. Complete strobila contains about 200 segments. Strongly serrated margins seen in anterior portion of strobila become gradually less evident and disappear toward the posterior end, where the gravid segments become sub-elliptical. Mature segments in contracted material measure about 1 mm. long by 2 mm. wide; in well relaxed strobilae, they measure up to 5 mm. long by 1 mm. wide. Scolex from 360 to 490 μ in diameter; not distinct from unsegmented neck of equal width. Rostellum and apical sucker absent. Suckers from 144 to 220 μ in diameter. Excretory canals of typical formation; ventral canal 32 μ in diameter, and dorsal canal 8 μ in diameter. Genital pores irregularly alternate, situated in anterior third of segment;

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position of genital pore relatively variable according to state of contraction of strobila. Cirrus sac claviform to pyriform, from 324 to 490 μ long by 144 to 194 μ wide in pre-gravid segments. Cirrus sac may or may not extend dorsally past the ventral excretory canal. Cirrus aspinose. Vas deferens somewhat coiled. Internal and external seminal vesicles absent. Testes, about 300 in number,² situated in two lateral fields posterior to ovary; lateral fields not joined by band of testes at posterior end of segment. Vagina posterior to cirrus sac; course of vagina perpendicular to margin of segment in contracted material and directed postero-medially in relaxed segments. Vagina is uncoiled tube, abundantly supplied with surrounding glandular cells. Enlargement of variable size near medial end of vagina serves as seminal receptacle. Much-branched vitelline gland poral, situated just posterior to proximal end of cirrus sac. Ovary highly branched and aporal, extending in well-relaxed material posteriad beyond anterior limits of vitelline gland. Uterine stem evident early in strobila. Form of gravid uterus typical for genus, with 30 to 40 lateral branches, often secondarily divided, on each side. Eggs spherical, from 17 to 28 μ in diameter.

Host: *Marmota caligata broweri* Hall and Gilmore.

Type Locality: Tolugak Lake, Arctic Alaska.

Habitat: Small intestine.

Type: A whole mount containing a complete strobila has been deposited in the Helminthological Collection of the U. S. National Museum, slide no. 47535.

DISCUSSION

There are at least 8 apparently valid species, all parasitic in rodents, previously assigned to the genus *Catenotaenia*, viz., *C. capensis* Ortlepp, 1940, *C. dendritica* (Goeze, 1782), *C. geosciuri* Ortlepp, 1938, *C. linsdalei* McIntosh, 1941, *C. lobata* Baer, 1925, *C. oranensis* Joyeux and Foley, 1930, *C. pusilla* (Goeze, 1782), and *C. rhombomidis* Shul'ts and Landa, 1935. Of these, the description for *C. rhombomidis* is not available, and the species is not considered further here.

Catenotaenia reggiae is differentiated by the large size, strongly developed strobila, and cirrus sac length. On the basis of excretory system (determined from whole mounts only), which is net-like in *C. lobata* and *C. capensis*, *C. reggiae* falls into the group with those containing simple excretory canals, viz., *C. pusilla*, *C. dendritica*, *C. oranensis*, *C. linsdalei*, and *C. geosciuri*. It is separable from all but possibly *C. capensis* on the basis of testes number. *C. reggiae* is characterized also by form of gravid uterus (Fig. 3), which differs from any of the others with which it has other characteristics in common. It is perhaps most nearly similar to *C. dendritica*, but from this it can be separated by the failure of the testicular fields to unite (in *C. reggiae*) at the posterior end of the segment, by the great difference in cirrus sac size, and by the character of the gravid uterus.

In various anoplocephaline genera, including *Catenotaenia*, the lack of constant morphological characters (e.g., rostellar hooks) makes it necessary to pay particular attention to variation when specific differentiation is attempted. In *Catenotaenia* it would seem particularly advisable to take into consideration the state of strobilar contraction, since the relative positions of organs are often greatly influenced by this. Any lot of material consisting solely of strobilae either uniformly contracted or relaxed could convey an impression which might lead to an altogether erroneous conclusion in regard to specific characterization. Joyeux and Baer (1945) have remarked on the morphological variation seen in *C. pusilla*. It appears that gravid segments in some cases may show specific features more readily recognized than those seen in mature segments alone.

Possible variation related to host occurrence has been considered, since other

² Testes so numerous that an accurate count is not possible.

species of the genus have been recovered from sciurid hosts, and host-specificity does not appear to be well developed. From North America only *C. dendritica* (erroneously reported by Rausch and Tiner (1948) as *C. pusilla*³) and *C. linsdalei* have been reported. Of these, only the former so far has been reported from sciurids. Although some of the material available for study is in poor state of preservation, all seems referable to *C. dendritica*. The writer has collected this species from *Sciurus niger rufiventer* Geoffroy and *Glaucomys sabrinus macrotis* Mearns in the United States, and from *Clethrionomys rutilus dawsoni* (Merriam) from Alaska. No differences have been observed in this material which might be attributed to modification by host occurrence. It is concluded that the morphological peculiarities exhibited by *C. reggiae* are clearly specific in nature.

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³ The probable error in this identification was called to the writer's attention by Dr. J. G. Baer, who pointed out the prevalence of *C. dendritica* in Nearctic sciurids.

EXPLANATION OF PLATE

Catenotaenia reggiae n. sp. Figs. 1, 2, and 3 were drawn with the aid of a projector. The scale has a value of 250 μ in figs. 1 and 4; 500 μ in fig. 2; 1 mm. in fig. 3.

FIG. 1. Scolex.

FIG. 2. Mature segment, from well-relaxed strobila.

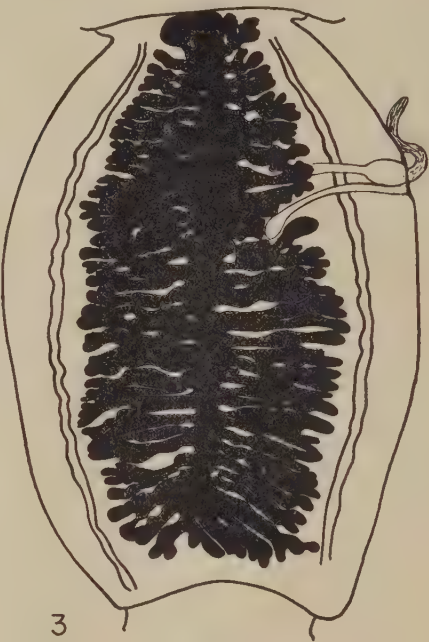
FIG. 3. Gravid segment.

FIG. 4. Semi-diagrammatic drawing of terminal region of genital ducts.

PLATE I



2



3



1



4

BIOMETRICAL NOTES ON THE HYBRIDIZATION OF *CULEX PIPIENS* L. AND *C. QUINQUEFASCIATUS* SAY

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Members of the *Culex pipiens* complex have been the subject of a number of hybridization studies (Weyer, 1936; Marshall, 1938; Roubaud, 1941; Farid, 1949; Sundararaman, 1949; Kitzmiller, 1950; Roubaud and Ghelelovitch, 1950). Sundararaman devised an objective criterion for comparing *Culex pipiens* with *C. quinquefasciatus*. He used a ratio DV/D where D was the measured distance between the tips of the dorsal arms of the phallosome and DV was an average of the distances from the outer side of the dorsal arms to the extreme tips of the ventral arms. He found the ranges of this ratio to be distinct in the two forms; that of *C. pipiens* being from 0.0–0.5 and of *C. quinquefasciatus*, 0.5–1.4. The ranges found in the present study are 0.0–0.2 and 0.6–1.1 respectively. Intermediate between the two parents, in Sundararaman's work, were experimentally produced hybrids, as well as some specimens, presumably hybrids, which were collected in the field.

The present authors have extended this analysis to the fourth generation hybrids and have also back-crossed hybrids with the parent strains. The source material was Sundararaman's colonies. The average DV/D ratio in the *C. pipiens* parent was $0.13 \pm .04^*$ (47 specimens) and $0.83 \pm .12$ (42 specimens) in the *C. quinquefasciatus* parent. The first generation hybrids, using the *C. pipiens* male, averaged $0.36 \pm .07$ (53 specimens). These hybrids were inbred for three more generations in which the ratios $0.37 \pm .12$ (152 specimens), $0.45 \pm .12$ (24 specimens) and $0.40 \pm .11$ (23 specimens) respectively were found. The reciprocal cross using male *C. quinquefasciatus* showed a ratio of $0.31 \pm .08$ (49 specimens) in the first generation and in the three following generations it was $0.38 \pm .14$ (79 specimens), $0.34 \pm .15$ (25 specimens), and $0.40 \pm .14$ (25 specimens) respectively. When the male hybrid from the *C. pipiens* female was crossed with the female hybrid from the *C. quinquefasciatus* female, the ratio in the offspring was $0.37 \pm .19$ (72 specimens). It can be seen that there was no overlapping of the parents in the DV/D ratio and that the hybrids were intermediate between the parents. The inbred generations of hybrids showed no tendency to revert to a parental form but seemed to show increased variability in later generations. Analysis was discontinued at the fourth generation but the hybrid colonies were maintained through 10 more generations with no indication of a decrease in vigor.

The hybrids obtained from crossing *C. pipiens* males and *C. quinquefasciatus* females were back-crossed to both parents. When the hybrids were mated to *C. pipiens* females the ratio in the offspring was $0.23 \pm .07$ (115 specimens); it was $0.31 \pm .14$ (80 specimens) when mated to *C. pipiens* males. It was $0.46 \pm .06$ (171 specimens) when mated to *C. quinquefasciatus* females and $0.60 \pm .16$ (96 specimens) when mated to *C. quinquefasciatus* males. In each case, the back-cross

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* Standard deviation.

was intermediate between the hybrid and the parent so that there was a shift in the DV/D ratio toward that of the parent. Some of these hybrids fall within the ranges exhibited by true *C. pipiens* or *C. quinquefasciatus* and such specimens found in nature would be unrecognized as hybrids. Farid (1949) and Sundararaman (1949) recommended that these two forms be considered subspecies, an idea which is corroborated by the present study. Specimens, presumably hybrid, have been found in nature (Sundararaman, 1949) but it is not known whether *C. pipiens* and *C. quinquefasciatus* represent overlapping populations or whether the entire complex is a cline with respect to this particular character.

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EVIDENCE OF DIRECT AMEBACIDAL ACTION OF AGENTS TESTED IN VITRO AGAINST *ENDAMOEBA HISTOLYTICA*

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Until very recently *in vitro* studies of amebacides and antibiotics against *Endamoeba histolytica* were performed with the ameba growing in association with one or more bacteria. This association creates the problem of indirect action of these drugs against the bacterial flora, which in turn may markedly reduce the amebic population. It has been postulated by Anderson and Anderson (1950) that perhaps some of the antibiotics act indirectly by inhibiting the bacterial flora of the intestinal tract. Balamuth and Brent (1949) reported direct amebacidal action of prodigiosin, based on the fact that the amebacidal agent did not affect the growth of the bacterial associate.

Recently Phillips (1950) and Phillips and Rees (1950) isolated a strain of *E. histolytica* in association with *Trypanosoma cruzi*. Our laboratory was able to obtain a culture of this ameba and we have employed it for the *in vitro* drug testing of some conventional amebacides and antibiotics against *E. histolytica* (Nakamura and Anderson, 1950a). A direct "amebacidal" end-point was determined for the agents tested. This was based on the fact that the *T. cruzi* was not susceptible to chemotherapeutic agents (von Brand, Johnson, and Rees, 1946; Kofoed, McNeil, and Wood, 1937). Our experiments indicated that amebacidal concentrations of these agents when tested against *T. cruzi* alone, showed no toxicity towards the trypanosomes. The two main criteria employed were growth and motility when subcultured into appropriate media. However, it was felt that these two criteria were inadequate for complete elimination of drug action on the trypanosomes. The present investigation reports on another criterion which may be used to show that the trypanosomes are not affected by the amebacides.

MATERIALS AND METHODS

The trypanosome material was prepared by growing *T. cruzi* on Chang's medium (Chang, 1947). The method of preparing the trypanosomes for the respiratory studies is described in another communication (Nakamura and Anderson, 1950b). To 9 ml. of the saline suspension of the cells, 1.0 ml. of 1% glucose solution was added. One ml. of this suspension was placed into the Warburg flasks. Into the side arms were placed concentrations of antibiotics and amebacides ranging closely to the determinations obtained by our drug testing procedure (Nakamura and Anderson, 1950a). Controls without antibiotics or amebacides had distilled water in the side arm. Six representative agents were tested, at their "amebacidal" concentrations, to study their effect on the oxygen consumption of *T. cruzi*. The agents tested were aureomycin, emetine, carbarsone, terramycin, polymyxin B, and bacitracin. All tests were made at 37.2° C. Manometric readings were recorded

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every 15 minutes for a period of three hours. The agents in the side arm were tipped in immediately following the 90-minute readings. The gas phase was air and the equilibration period 10 minutes. The manometers were shaken 100 times per minute.

The trypanosomes were examined after the respiratory studies to check their motility.

RESULTS

The graphs in Figure 1 indicate the results obtained from the respiratory determinations. As can be seen, the oxygen uptake of the trypanosomes was not appreciably affected by the levels of the agents that showed "amebacidal" action. Microscopic examinations indicated also that the trypanosomes were actively motile after 3 hours.

DISCUSSION

Another criterion is available for determining whether or not the amebacidal agents tested and found to be toxic to the ameba (Nakamura and Anderson, 1950) are toxic to the trypanosomes. The two previous criteria employed were growth upon subculture and motility after contact with the drugs. Since the amebacides and antibiotics examined do not affect the trypanosomes, it may be said that the "amebacidal" level determined by our *in vitro* evaluation indicates a direct amebacidal action and that it was not due to an indirect action on the associated trypanosomes.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Charles W. Rees, National Institutes of Health, Bethesda, Maryland, for furnishing us with the Culbertson strain of *T. cruzi* used; to Mrs. M. James, Mrs. B. Mythen, and Miss D. Swanman for their technical assistance.

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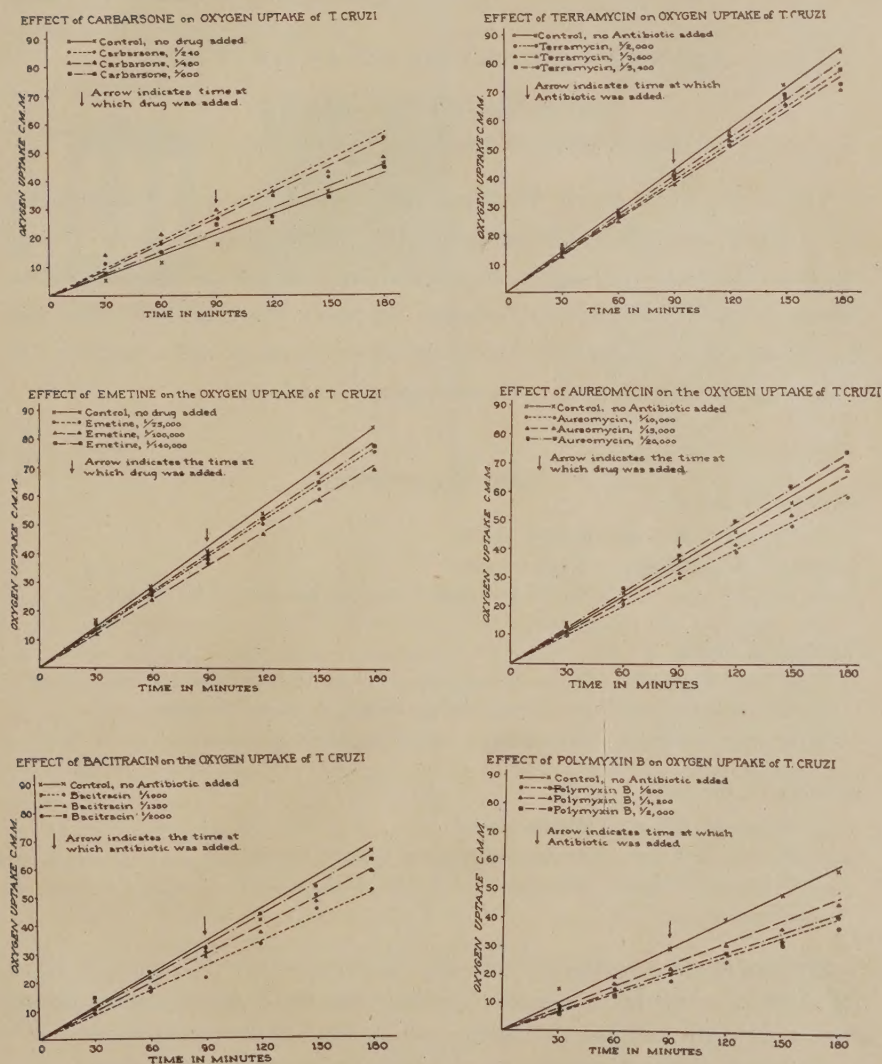


FIG. 1.

AMERICAN SOCIETY OF PARASITOLOGISTS

TWENTY-SIXTH ANNUAL MEETING

Chicago, Illinois, November 15th, 16th, 17th, 1951

The Twenty-Sixth Annual Meeting of the American Society of Parasitologists will be held in conjunction with the meetings of The American Society of Tropical Medicine, The American Academy of Tropical Medicine and the National Malaria Society, on Thursday, Friday and Saturday, November 15th-17th, 1951. The Congress Hotel will be the headquarters for these joint meetings, and a tentative program for the American Society of Parasitologists is as follows:

TENTATIVE PROGRAM

November 15, Thursday

- 9:00 a.m.-12:00 noon. Regular session
- 2:00 p.m.- 5:00 p.m. Regular session
- 7:00 p.m. Dinner and council meeting, members of the council

November 16, Friday

- 9:00 a.m.-11:00 a.m. Symposium on "The Ecology of Vectors of Parasitic Diseases"
- 11:00 a.m.-12:00 noon. Presidential address by Dr. Benjamin Schwartz
- 2:00 p.m.- 5:00 p.m. Demonstration session at Roosevelt College

November 17, Saturday

- 9:00 a.m.-11:00 a.m. Regular session
- 12:00 noon- 1:30 p.m. Annual luncheon and business session
- 2:00 p.m.- 5:00 p.m. Joint session, A.S.T.M., N.M.S., A.S.P.

Previous meetings held jointly with The American Society of Tropical Medicine, The American Academy of Tropical Medicine and the National Malaria Society have yielded very stimulating programs, and all members are urged to attend this Twenty-Sixth Annual Meeting of the American Society of Parasitologists.

Respectfully,

H. W. BROWN, *Secretary*
